GASTRO-ENTERITIS

A BACTERIOLOGICAL AND CLINICAL STUDY OF THE PATHOGENICITY OF ESCHERICHIA COLI

A Thesis submitted to Glasgow University for the Degree of Doctor of Medicine

by

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December, 1957.

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ACKNOWLEDGEMENTS:

I wish to record my thanks to Dr. John S. Stevenson, Stobhill General Hospital, Glasgow, for introducing me to the subject of this thesis and for his continued interest and advice during the course of my investigations. Other medical colleagues to whom I am especially indebted are acknowledged in the text. My thanks are also due to the medical staffs of Stobhill General and Ruchill Hospitals, Glasgow, for providing access to patients and case records and for their co-operation in sending specimens for examination.

C O N T E N T S

INTRODUCTION

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The aetiology of many of the diarrhoeal diseases of infancy is imperfectly understood. Nevertheless, classification of these diseases is usually made empirically on an aetiological basis. That outlined by Laurent (1944) may be taken as a typical and generally acceptable one, in which infantile diarrhoea is divided into three main groups.

1. Non-infective diarrhoea, which usually occurs as a result of faulty feeding or as an accompaniment of dentition.

2. Symptomatic diarrhoea, which occurs in association with parenteral infections especially those of middle ear and the upper respiratory tract.

3. Infectious diarrhoea of primary gastro-intestinal origin. This group comprises infections of known aetiology such as the dysenteries, enterica and salmonella infections, and also those of unknown or doubtful aetiology.

Nowadays, the term epidemic infantile gastro-enteritis is commonly used to denote the latter type of infectious diarrhoea and, during the last decade, interest has centred on the part played by certain serological types of Escherichia coli in the aetiology of this condition.

From early in 1949 it had been apparent that strains of E. coli similar to those associated with infantile gastro-enteritis cases elsewhere in Britain, were occasionally being isolated from infant and adult patients in Stobhill General Hospital, Glasgow. At that time the significance of the findings was not clear.

Accordingly, in October, 1951, an investigation was begun, the aims of which were as follows:-

1. To note the occurrence of certain specific serological types of *E. coli* among cases of gastro-enteritis in the hospital.

2. To record serological and/or biochemical variations within each type in order

   (a) To assess the relationship of such variations to pathogenicity.

   (b) To investigate and develop methods suitable for the routine isolation and typing of *E. coli* strains from faeces.

3. To determine from clinical records and by experimental procedures the pathogenicity of specific serological types of *E. coli* for human hosts.

This thesis records the results of the investigation in respect of the following serological types of *E. coli*, viz., O111 B4, O55 B5, O26 B6, O125 B15, O126 B16 and O86 B7.

Other serological types have occurred in association with epidemics of gastro-enteritis since the commencement of this study. Such strains were included in the investigation as they became available, but they were isolated only very infrequently or not at all and have, therefore, not been referred to in the results.

CHAPTER 1

GENERAL REVIEW OF LITERATURE

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The concept of *E. coli* as an aetiological agent of epidemic infantile gastro-enteritis though topical is not an entirely new one. As early as 1912 Bahr and Bahr and Thomsen tried to show a relationship between strains of *E. coli* isolated from new-born calves suffering from diarrhoea and those recovered from infants with diarrhoea. The organisms were classified by fermentation reactions and it was found that strains from both sources were of the same fermentation types. In 1923 and 1927, Adam, again by fermentation reactions, attempted to classify coliform organisms obtained from cases of infantile gastro-enteritis. Adam believed that certain strains of *E. coli* caused this disease and that these toxic strains had a different fermentation pattern from other non-toxic strains.

In 1937, Blacklock, Guthrie and McPherson carried out post mortem examinations on babies dying of primary acute non-specific gastro-enteritis in a Glasgow children's hospital. They found coliform organisms present in the duodenum and upper small bowel in these cases but only rarely and in small numbers in control non-diarrhoeal patients. It was suggested from those findings that the virulence of *E. coli* may have been enhanced to such an extent by growth in an unusual part of the bowel that the organism had become an infective agent. Payling Wright and Payling Wright (1946)

having studied the death rates from gastro-enteritis in Willesden during the years 1930 to 1938, were of the opinion that a significant proportion of the deaths were in some degree associated with each other in time or place or both and suggested that this distribution might have resulted from the widespread dissemination throughout the community of one or more strains of some common micro-organism of relatively low virulence for all but the infant population. This point of view is in accord with the hypothesis that E. coli may exert enteropathogenic effects in infancy.

Recent advances in our knowledge of the role of coliform bacteria in infantile gastro-enteritis date from the observations of Beavan (1944) and Bray (1945). Beavan noted that the stools of infants with severe diarrhoea often emitted a characteristic seminal odour which, on culture of faeces, proved to be due to the presence of certain strains of E. coli. These strains were identified as E. coli type neapolitanum, which term was held to signify a coliform organism which normally fermented sucrose and salicin but only slowly fermented maltose. Further investigation showed that organisms having these characteristics were frequently found in cases of infantile gastro-enteritis and only rarely in unaffected infants. Bray (1945), in England, studied fifty-one cases of infantile diarrhoea between 1943 and 1944. Thirty-nine cases were severe and twenty died. All infants were under one month old and all were bottle fed. A rabbit antiserum was prepared against a

typical, seminal smelling, late maltose fermenting strain of E. coli isolated from one affected infant. This antiserum agglutinated living unheated coliform cultures from the stools of affected infants but had no effect on strains isolated from other sources. Forty-two of forty-four cases of summer diarrhoea produced strains agglutinable by this antiserum while only four such strains were obtained from one hundred normal infants. Post mortem examinations carried out on fatal cases were remarkable only for the paucity of specifically abnormal findings and attempts to reproduce the disease in lactating mice, kittens and rabbits were uniformly unsuccessful. These investigations led Bray to postulate that a single serological type of E. coli had been isolated from his gastro-enteritis patients and that this type was uncommon among normal infants. He did not consider, however, that the organism had been proved to be the cause of gastro-enteritis.

In association with Beavan, Bray in 1948 recorded further results using the antiserum already referred to. Slide agglutination was performed on cultures from forty infants with diarrhoea and from eighty control infants. An organism, apparently identical with that isolated from the first study, was obtained from thirty-five infants with diarrhoea and from only three control infants. The identity of the E. coli strains isolated was later confirmed by tube agglutination tests. The authors pointed out that in severe cases it was usual to isolate E. coli neapolitanum in apparently pure culture.

The importance of the observations of Beavan and Bray received early recognition and further investigative work by paediatricians and bacteriologists has substantially confirmed these observations. It has also become apparent since 1948 that strains of E. coli other than those described by Bray and Beavan have been implicated from time to time in institutional outbreaks of infantile gastro-enteritis. Much of the subsequent research work has been directed to establishing proof of a direct aetiological relationship between these strains and the disease.

In 1948, Giles and Sangster reported on ninety-three cases of primary infective gastro-enteritis in infant patients in the City Hospital, Aberdeen. No recognised bacterial or viral pathogen was isolated from ninety-two of these cases but over 90% yielded an organism closely resembling that described by Bray. All strains isolated were non-motile and were serologically and biochemically identical with each other. There was evidence of case to case spread in the wards concerned, with sudden onset of illness after an incubation period of three to ten days. A case fatality rate of 56.5% gives an indication of the severity of the disease. Post-mortem examinations on fatal cases revealed no specific abnormalities. Attempts to reproduce the disease in new-born mice and kittens both by feeding and by rectal injection of cultures of the organism were unsuccessful.

A second report from Aberdeen by Giles, Sangster and Smith (1949) recorded two hundred and seven cases with diarrhoea of

uncertain aetiology admitted during 1947 to the City Hospital. E. coli neapolitanum serologically identical with Bray's organism was isolated from 95% of affected infants, one hundred and five of whom died. The organism was isolated from only thirteen of seven hundred and twenty-one controls. The authors drew attention to the chronological relationship between presence of organism and appearance of diarrhoea.

In November, 1947, a further strain of E. coli began to be encountered in cases of infantile diarrhoea in Aberdeen. This organism was motile, did not ferment salicin and was serolcgically distinct from the strains isolated earlier in the year. It was named E. coli neapolitanum type beta to distinguish it from the alpha type previously encountered. The beta type was isolated from the stools of twenty-one of forty-eight cases of infantile diarrhoea but from only three of fifty-three healthy infants and from one of seventy-four healthy adults.

In 1947 and 1948 Taylor, Powell and Wright (1949) carried out a clinical and bacteriological investigation of a number of epidemic and sporadic cases of infantile diarrhoea in England. None of the recognised pathogenic enteric organisms was isolated in over one hundred cases of infantile diarrhoea. Studies for virus in four fatal cases were negative. However, a coliform organism of a single homogeneous serological type was isolated frequently from rectal swab cultures. This organism was named D.433. It was first identified during an outbreak of diarrhoea in the newborn

nursery of a maternity unit in which nine infants were involved. All gave positive stool cultures of this organism. The same serological type was again isolated from all twenty-two babies with diarrhoea who were examined during two outbreaks in a mother and baby hostel and one outbreak in a residential nursery. Thus a constant relationship between E. coli D.433 and cases of infectious infantile diarrhoea was shown.

The organism was also isolated from twenty-one of twenty-five infants with intercurrent diarrhoea during treatment for other conditions in a children's hospital. In patients from two further units, one group being consecutive admissions to the gastro-enteritis ward of a children's hospital and the other, infants admitted to an infectious diseases hospital, six of fourteen and five of twenty-four cases yielded D.433.

As a control group one hundred and seventy-five normal babies attending infant welfare clinics in London and residential nurseries in Hertfordshire were examined. All were negative for E. coli type D.433. Similar findings were present in the case of thirty-three babies admitted to a London Hospital for diseases other than diarrhoea. Investigation of a further thirty-four babies who had been in contact with cases yielding D.433 showed that nine of the thirty-four were excreting the organism. None, however, had diarrhoea. All the above institutions were re-investigated after intervals of several months. Eighty-two infants had rectal swabs taken which were negative for E. coli D.433.

The distribution of this organism was not confined to one area or one season of the year. It was found in infants ranging from ten days to eleven months, but the majority were under five months old. It is interesting to note that only two of the babies from whom *E. coli* D.433 was obtained were wholly breast fed.

E. coli D.433 was identified by Kauffmann and Dupont (1950) as being of the same antigenic type as the strains of *E. coli neapolitanum* recovered by Bray (1945) and Giles *et al.* (1948, 1949). The organism was classified by its somatic 'O' and surface 'B' antigens and designated *E. coli* O111 B4. The beta variety of *E. coli neapolitanum* described by Giles *et al.* (1949) was shown to be of serological type 055 B5. Kauffmann and Dupont at this time were not prepared to take any definite stand concerning the aetiological relationship of these strains to infantile gastro-enteritis.

Nevertheless, further evidence implicating one or other of these two types was steadily being built up. Smith (1949), continuing the Aberdeen investigation, reported on seventy-five cases of suspected infantile gastro-enteritis occurring in a hospital cubicled unit. Sixty of these patients (81%) yielded type O111 B4 or 055 B5 with again failure to isolate either organism from large control groups of patients. In Birmingham, Rogers, Koegler and Gerrard (1949) found eighty-six babies in the Children's Hospital harbouring *E. coli* O111 B4. Twenty-five of these babies remained

symptomless and sixty-one either had or developed symptoms of gastro-enteritis. Treatment with streptomycin was tried in twelve cases but did not appear to be efficacious. Twelve further cases were successfully treated with chloramphenicol. This antibiotic, which was effective *in vitro* against *E. coli* 0111 B4, cleared stools of the organism within four days and though there were three bacteriological recurrences, no case relapsed clinically. Similar clinical improvement was noted in eight cases of gastro-enteritis by Magnusson *et al.* (1950) following the use of aureomycin. The treated babies were harbouring *E. coli* 0111 B4 and clinical improvement corresponded with elimination of the organism from stools. This good response to antibiotic therapy was further evidence in favour of the aetiological relationship of the type 0111 B4 to infantile gastro-enteritis.

From Liverpool, Kirby, Hall and Coackley (1950) confirmed the epidemiological findings of others in respect of *E. coli* 0111 B4. These authors extended previous observations, however, to include feeding experiments on adult volunteers. Cultures of *E. coli* 0111 B4 were fed to six adults, three of whom developed diarrhoea and abdominal discomfort. Neter and Shumway (1950) also reported at this time successful production of gastro-enteritis in a two month old infant by causing it to ingest approximately one hundred million viable organisms of type 0111 B4. It is obvious that such an experiment is not practicable on a large scale but Smith, Galloway and Speirs (1950) suggested that the closest approximation

to crucial feeding experiments on infants was the study of the effects of cross infection by strains of E. coli. In a series reported by these workers it was shown that cross infection with E. coli 055 B5 definitely prolonged illness, as judged by duration of stay in hospital.

Smith et al. (1950) also tested for O and H agglutinins in two serum samples from each of twenty-nine infants harbouring E. coli 055 B5 H6. The first sample was obtained on admission to hospital and the second during convalescence some fifteen to twenty-five days later. In eight of the twenty-nine cases, agglutinins to the 055 or H6 antigens were demonstrated in the convalescent specimen. The increase in titres obtained between admission and convalescent specimens was so small, however, that no definite aetiological relationship of E. coli 055 B5 to gastro-enteritis could be proved by this means.

Thus, by 1950, attempts to assign a definite aetiological role to specific E. coli sero-types had been made along certain well recognised lines, viz.,

1. By isolation of specific serological types of E. coli from high proportions of cases of nosocomial epidemic infantile gastro-enteritis with clinical and bacteriological evidence of case to case spread.

2. By feeding experiments on human volunteers.

3. By investigating clinical responses of patients to treatment with antibiotics effective against E. coli.

4. By investigating serological responses in affected individuals.

5. By feeding experiments on laboratory animals.

Most of the research work on the subject subsequent to 1950 has continued to follow one or more of these lines. From an epidemiological point of view additional evidence has been forthcoming in the work of Rogers (1951) who demonstrated spread of specific serological types of E. coli by articles in common use in paediatric wards, and by air, dust and nursing manipulations. Rogers and Koegler (1951) have traced, bacteriologically and clinically, case to case spread from one institution to another. From Glasgow, further epidemiological work implicating serotypes 0111 B4 and 055 B5 in cases of gastro-enteritis was reported by Ross (1952) and by Shanks and Studzinski (1952). The work of Kauffmann and Dupont (1950) had indicated that strains of E. coli 0111 B4 and 055 B5 could be subtyped antigenically by investigation of flagellar antigens. Epidemiolcgical studies on this basis were recorded in 1953 by Smith, for strains of 0111 B4 and 055 B5, and by Wright and Roden for strains of 055 B5. French workers had previously shown the possibility of applying phage typing methods to investigating epidemic strains of E. coli (Nicolle et al., 1952). In 1954, in Brighton Children's Hospital Jameson et al. recorded a lengthy experiment in a 28-cot non-cubicled children's ward. E. coli 0111 B4 or E. coli 055 B5 were isolated from seventy-four of seventy-seven cases of gastro-enteritis occurring in the ward. E. coli 055 B5 was shown to be serially transmitted from one admission to one hundred and thirty-seven other patients over a period of seventeen months. Sixty-eight of

these patients developed symptoms of gastro-enteritis and thirteen died. By reducing the number of cots in the ward from twenty-eight to twenty, by adoption of a rigid barrier nursing technique, pasteurisation of all feeds and careful vetting of admissions, in particular the exclusion from the ward of any child known to have had a previous attack of gastro-enteritis, nosocomial diarrhoea was eliminated for a period of sixteen months. Anderson, Crockatt and Ross (1954) studied the epidemiology of infantile gastro-enteritis in three separate wards in two of Glasgow's infectious diseases hospitals. They found that despite variations in nursing efficiency similar cross infection rates for specific serological types of E. coli were obtained for each ward. Of one hundred and thirteen cross infections with strains of E. coli, seventy-six (67%) were associated with symptoms of varying severity and five babies died. It was shown by these workers that cross infection rates were directly affected by the number of E. coli positive cases admitted to a ward, by the number of babies at risk in a ward, and by the duration of stay in hospital.

In a report of a five year investigation of infantile gastro-enteritis carried out by Hutchinson (1957) in Southamptom the sero-types 055 B5 and 0111 B4 were found to be more frequently isolated from infants in epidemic than in non-epidemic periods. Though many cases were found to harbour these serotypes, symptomless carriers did occur frequently and in all age groups. It was suggested that carriers in infancy and childhood might be responsible for

maintaining a low-grade type of infection in a community. The pattern of the outbreaks encountered by Hutchinson suggested a gradual enhancement of virulence of E. coli 0111 B4 and 055 B5 with local spread at first followed by wide dispersal throughout the district and then gradual reversion to a less invasive type of illness.

With regard to feeding experiments in adult volunteers, two large investigations have been reported since 1950. Ferguson and June (1952) fed a mixture of three strains of E. coli 0111 B4 obtained from diarrhoeal infants to forty-six healthy young adult male volunteers. The organisms were incorporated in milk in numbers varying from 9,000 x 10^6 to 7 x 10^6 per feed. Those volunteers receiving high dosage of organisms developed diarrhoea, anorexia and other toxic symptoms whereas symptoms tended to be mild or absent when dosage of organisms was relatively small. Forty-five controls who were fed milk only developed no symptoms. Most of the volunteers in the test group had O agglutinins to E. coli 0111 B4 demonstrable in post-feeding specimens. A further control experiment in which twelve volunteers were fed 9,000 x 10^6 organisms of a "normal" strain of E. coli produced no symptoms and no serum agglutinin responses. A second experiment on the same lines using a mixture of three strains of E. coli 055 B5 was recorded by June, Ferguson and Worfel (1953). Similar results were obtained though the symptom complex developed from ingestion of E. coli 055 B5 was found to be milder than that obtained with

0111 B4.

A few of the volunteers in these feeding experiments became symptomless carriers of one or other strain of E. coli. Stevenson (1950, 1952) and McNaught and Stevenson (1953), however, have shown that strains of E. coli 0111 B4, 055 B5 and 026 B6 do from time to time occur in association with diarrhoea in adults, frequently in those debilitated by other disease or old age.

While the greater part of the researches cited has dealt with the occurrence of serotypes 0111 B4 and 055 B5 in epidemics of infantile gastro-enteritis in Britain, reports along similar lines regarding one or other of these types have been received from Holland (Beeuwkes et al., 1949), Sweden (Magnusson et al., 1950), Denmark (Kauffman and Dupont, 1950), U.S.A. (Neter and Shumway, 1950, Modica et al., 1952), Palestine (Drimmer-Herrnheiser and Olitzki, 1951), Germany (Braun and Henckel, 1952), Canada (Alimanestianu-Butas et al., 1953), France (Buttiaux et al., 1951), Ireland (Holland, 1951), Japan (Hiroki, 1951) and Hungary (Rauss and Ujvary, 1953).

With regard to other experimental aspects dealing with serum agglutinin responses to coliform strains, the therapeutic effects of antibiotics and experiments on laboratory animals, it is proposed to refer to published work on these subjects in Chapters 4, 5 and 6 respectively.

Although most attention has been paid to the serotypes 0111 B4 and 055 B5 several other types have been isolated from

sporadic cases and epidemics of infantile gastro-enteritis. Orskov (1951) investigated strains of E. coli 026 B6 received at the State Serum Institute, Copenhagen. Several of these strains had been isolated from cases of infantile diarrhoea in England and Finland, and Orskov suggested that type 026 B6 might have a relationship to gastro-enteritis similar to that of types 0111 and 055. In 1953, Smith recorded the isolation of 026 B6 from twelve cases of infantile gastro-enteritis of unspecified severity and from two carriers. McNaught and Stevenson (1953) noted the occurrence of this type in the stools of eight adults, seven of whom had diarrhoea. In the investigation of Anderson, Crockatt and Ross (1953) already referred to, five of nine infants harbouring E. coli 026 B6 had mild gastro-intestinal symptoms. The organism was also isolated from thirteen babies in a residential nursery by McDonald and Charter (1956), but all cases remained symptomless.

In 1952, Taylor and Charter described outbreaks of infantile gastro-enteritis associated with E. coli type canioni, now designated 0125 B15. McDonald and Charter (1956) also reported the occurrence of E. coli 0125 B15 in a small number of institutional gastro-enteritis cases. The organism was found by McNaught (1956) in the stools of sixty infants in the paediatric wards of a Glasgow Hospital. Twenty of these infants had diarrhoea but the clinical effects associated with E. coli 0125 B15 appeared to be much less severe than those associated with E. coli 0111 B4 and 055 B5.

A further serological type, viz., O126 B16, was investigated by Charter and Taylor (1952). This type was isolated from only a small number of infants and associated diarrhoeal symptoms were of a mild nature. Similar findings with this organism were referred to by McNaught (1956). McDonald and Charter (1956) also noted the occurrence of E. coli O126 B16 in the stools of thirteen infants, none of whom, however, had diarrhoeal symptoms.

Smith (1953) extending observations on the epidemiology of infantile gastro-enteritis in Aberdeen isolated E. coli O119 B14 from eleven cases of gastro-enteritis and from ten of twenty-five contacts of these cases. No further published evidence on the clinical effects of this organism is available to date.

E. coli O86 B7, first designated by Taylor and Charter (1952) as type E990 has been associated from time to time with cases of infantile gastro-enteritis in England, Germany, Austria, Norway and Denmark (Orskov, 1954a). Braun and Reseman (1952) stated that this serotype behaved like O111 B4 and O55 B5 in epidemics of the disease. These workers were also able to reproduce severe gastro-enteritis in adults by feeding with cultures of E. coli O86 B7.

In a survey carried out in Palermo during 1949, Cefalu and Brancato (1953) found E. coli O group 25 to be frequently associated with cases of gastro-enteritis. Orskov (1954b) also refers to the isolation of this serotype from cases occurring in Rostock.

The occurrence of E. coli O127 B8 in an epidemic of infantile gastro-enteritis in Cincinnati, U.S.A., has been described

by Cooper *et al.* (1955). Forty-three of the forty-four patients whose rectal swabs were positive, had diarrhoea. Four affected infants died.

Two further types of *E. coli*, namely, O128 B12 and O114, have recently been described. Taylor and Charter (1955a) and Rogers *et al.* (1955) noted the occurrence of small epidemics and sporadic cases of gastro-enteritis in England and in Scotland associated with the former organism. *E. coli* O114 is held by Rogers and Cracknell (1956) to have accounted for small epidemics of the disease in the Birmingham area over several years. The type of illness and pattern of its spread in hospital are regarded by these workers as being similar to those associated with other more generally recognised types of *E. coli*.

In summation, the literature reviewed indicates that the greater part of the definitive work on the role of *Escherichia coli* in epidemic infantile gastro-enteritis has concerned the sero-types O111 B4 and O55 B5. Strong evidence exists for regarding these types as aetiological agents of the disease. Later work has shown that strains other than O111 B4 and O55 B5 have been associated with cases and epidemics of the disease in different parts of the world, though their pathogenic significance is not so well established.

CHAPTER 2

BACTERIOLOGY OF ESCHERICHIA STRAINS

I. THE ISOLATION AND IDENTIFICATION OF *E. COLI* SEROTYPES.

II. METHODS FOR PRODUCING FLAGELLATED FORMS OF *E. COLI*

III. SELECTIVE ISOLATION OF SPECIFIC *E. COLI* SEROTYPES FROM FAECES.

I. THE ISOLATION AND IDENTIFICATION OF E. COLI SEROTYPES

In October, 1951, three serological types of E. coli, viz., 0111 B4, 055 B5 and 026 B6, were known to be associated with cases and epidemics of infantile gastro-enteritis. In March, 1952, Dr. Joan Taylor, in a personal communication, suggested that three further types, of undetermined pathogenicity, be investigated. These were the serotypes 0125 B15, 0126 B16 and 086 B7 (types Canioni, E611 and E990 of Taylor and Charter, 1952). In a survey carried out in Stobhill General Hospital, Glasgow, from October, 1951, to March, 1954, the occurrence and clinical effects of these six serological types of E. coli were noted. The present section deals with the isolation and identification of E. coli strains in the survey.

The identification of specific types of E. coli in faecal cultures is largely based on a knowledge of the antigenic structure of these organisms. An account of the serological characteristics of Escherichia strains is, therefore, relevant.

Serological characteristics of the Escherichia Group:

The serological classification of the Escherichia group is based on the identification of somatic, surface and flagellar antigens, (O, K and H antigens). A full description of these antigens is given by Kauffmann (1947). Their characteristics may

be summarised thus:-

The somatic or 'O' antigens of E. coli are thermostable, resisting moist heat at 100°C. They are not destroyed by alcohol. Chemically they are mainly carbohydrates.

The term 'K' antigen as applied to E. coli covers a group of surface or capsular antigens designated L, A or B. Strains possessing L or B antigens are generally non-capsulated, whereas strains with A antigens possess capsules. The effect of these antigens is to render living organisms inagglutinable by their homologous O antisera.

The L, A and B surface antigens differ from one another in their heat resistance, in their effect on O agglutinability and in their agglutinin-binding capacity.

L antigens are thermolabile surface antigens which inhibit O agglutinability of the living organism. This O inagglutinability is destroyed by heating the bacteria at 100°C. for 1 hour. The agglutinin-binding capacity of the L antigen is also destroyed at this temperature.

A antigens are thermostable capsular antigens which also render organisms O inagglutinable. A antigens are destroyed by a temperature of 120°C. for 1 hour. The agglutinin-binding capacity is, however, thermostable.

B antigens are thermolabile surface antigens again giving rise to O inagglutinability. This capacity to interfere with O agglutination is destroyed by moist heat at 100°C., but the

agglutinin-binding property of B antigen is thermostable. The great majority of the strains of E. coli, which have been associated with gastro-enteritis, possess the B type of surface antigen.

Not all serological types of E. coli are motile. Flagellar antigens are present in motile strains. Many organisms on first isolation appear to be non-motile and H antigens are poorly developed. The H antigens of E. coli are monophasic.

The preparation and use of antisera:

The preparation of O, B and H antisera in the present investigation followed closely the methods described by Kauffmann (1954) and Charter and Taylor (1952). In practice it was found unnecessary to prepare separate O and OB antisera for individual strains. OB antisera sufficed, the O and B antigens being demonstrable in single antisera by testing boiled and live cultures respectively. This has also been the experience of Taylor (1955, personal communication) and is evident from results recorded by Charter and Taylor (1952).

Preparation of OB antisera:

Stock cultures of E. coli serotypes O111 B4, 055 B5, 026 B6, 0125 B15, 0126 B16 and 086 B7 were kindly supplied by Dr. Joan Taylor.

For the preparation of OB antisera each stock strain was inoculated on to a dried plate of 2% nutrient agar containing 0.1% glucose and incubated for 18 hours at 37°C. The growth was washed

off with 0.85% saline and the suspension of organisms adjusted to an opacity of 1000 x 10^6 per ml. Immediately following preparation, the suspension of live organisms was injected intravenously into an adult rabbit (weight approximately 2 kg.). The initial dose employed was 0.25 ml. followed at 3 to 5 day intervals by 0.5 ml., 1.0 ml., 1.5 ml. and 2 ml., giving a total of 5 injections. One week after the final injection the animal was test bled from an ear vein. If the serum gave satisfactory titres of the O and B antigens the animal was bled out on the following day. If not, a further injection of 2.0 ml. of suspension was given. The minimum titres accepted were B : 1 in 400 and O : 1 in 3,200. Titres of antisera prepared during the survey ranged from 1 in 400 to 1 in 1600 for the B antigen and from 1 in 3,200 to 1 in 12,800 for the O antigen.

In titration of antisera for demonstration of B antibodies, a suspension of living organisms in N/1 saline was obtained as outlined in the preparation of OB antiserum. The opacity was adjusted to that of an 18 hour broth culture. 0.25 ml. serial dilutions of OB antiserum were made in 3" x $\frac{1}{2}$" round bottomed test tubes. 0.25 ml. amounts of live suspension were added so that the final volume in each tube was 0.5 ml. The dilutions ranged usually from 1/50 to 1/6400 in geometric series. The tubes were incubated in a water bath for 2 hours at 37°C., left at bench temperature overnight, then examined using an X3 hand lens. Agglutinated particles were large white floccules and the suspending saline clear.

A similar procedure was adopted for demonstration of O antibodies in serum. In this case the bacterial suspension was boiled in a water bath for 1½ to 2 hours, the serum dilutions ranged from 1/200 to 1/25600, and incubation was carried out at 50°C. for 20 hours. The agglutinated particles were finely granular in appearance.

Preparation of H antisera:

H antisera were prepared from motile strains of E. coli. Antisera were made to H antigens 2, 6, 7, 11, 12, 13, 19 and 34. Stock strains of E. coli possessing these antigens were again supplied by Dr. Joan Taylor.

A highly motile 18 hour broth culture of each stock strain was obtained, as described in Section II of this chapter. The culture was standardised to an opacity of 1000 x 10^6 organisms per ml. and commercial formalin added to a final concentration of 0.25%. This formalised culture was used as an inoculum for intravenous injection of rabbits and preparation of the H antiserum proceeded as for OB antiserum. Titration of the H antiserum was performed against the inoculum by incubating at 50°C. for 2 hours. The minimum titre accepted was 1 in 12,800. Titres obtained varied from 1 in 12,800 to 1 in 102,400. The agglutination pattern was typically large, fluffy and dispersable on shaking.

Isolation of E. coli from faecal specimens:

During the present investigation, the method of isolating coliform organisms from faecal specimens and rectal swabs was similar to that employed by Charter and Taylor (1952) and now generally adopted as standard.

Charter and Taylor used three culture media in their investigations, viz., 5% horse blood agar, MacConkey agar and Desoxycholate-citrate agar. The former two were for the isolation of E. coli and the latter for demonstration of Salmonella and Shigella types. Pilot experiments in the present series showed that blood agar had no advantages over MacConkey in the isolation of coliform organisms provided that incubation was continued for 24 hours or less. Beyond this period rough variants tended to develop on MacConkey plates much more readily than on blood agar medium. Moreover, as adults and older children were included in the present survey it was found that many of the blood agar plates from such patients were overgrown with Proteus strains and quite unsuitable for coliform typing. Accordingly, it was decided to omit the blood agar plates, but ensure that MacConkey plates were examined after 18 hours' incubation. The procedure finally adopted was that all rectal swabs and faecal specimens were inoculated directly on to MacConkey, Desoxycholate-citrate and Difco S.S. agar plates. In addition a Selenite-F enrichment broth was also used. Sub-cultures from the Selenite-F broth were made to MacConkey plates after 18 hours' incubation. This system covered the isolation of E. coli and

members of the Salmonella and Shigella groups.

The further typing of E. coli strains from MacConkey medium was accomplished by testing five separate lactose-fermenting colonies and the inoculation "well" by slide agglutination using a pooled OB antiserum. This antiserum covered the six strains under investigation. The organisms were emulsified in a drop of saline on a glass slide and a loopful of undiluted pooled antiserum was added. In positive cases agglutination was prompt, the agglutinated particles usually large and curd-like and the suspending fluid clear. Varying degrees of non-specific agglutinations were frequently met with. In these cases the agglutination was longer in appearing, the particles were usually fine and the suspending fluid opaque. Moreover, all colonies showing any degree of agglutination were slide tested by the same method using a 1 in 500 watery solution of acriflavine instead of antiserum. The serotypes under investigation did not agglutinate in acriflavine, whereas many of the organisms showing doubtful agglutination were clumped by the acriflavine, indicating commencing rough variation (Pampana, 1933).

Colonies showing agglutination with pooled antiserum were re-tested using OB antisera prepared against the individual strains under investigation. Slide agglutinations were again performed, this time with the individual antisera at a dilution of 1 in 3 in order to cut down the incidence of non-specific agglutination. Strains were provisionally typed on the result of the slide agglutination and a report was issued to the ward.

These results were confirmed by setting up tube agglutinations with OB antiserum using live and boiled cultures of the organism as antigens for the B and O agglutinations respectively. The technique is identical to that already described in the titration of these antisera.

For H antigen determination, a representative colony of the strain under test was subcultured to induce motility, in the way described in Section II of this chapter. When satisfactorily motile cultures were obtained they were formolised and tested against the relevant H antisera, as already detailed for the titration of H antisera.

Biochemical Investigations:

When a serologically typeable strain of E. coli was isolated from any patient for the first time, one colony representative of that strain was retained for investigation of its biochemical characteristics. Fermentation reactions on the following substances were noted:-

> Glucose, lactose, mannite, sucrose, maltose, dulcite, sorbitol, salicin, adonitol, inositol, arabinose and xylose.

The medium for fermentation tests consisted of peptone water to which fermentable substance was added to a concentration of 1% (0.5% in case of dulcite). 1% Andrade's indicator was incorporated to detect acid change. The tests were incubated at 37°C. and were examined daily for a period of 30 days.

Additional tests, viz., Indole, H_2S and urease production,

citrate utilisation, liquefaction of gelatin, Voges-Proskauer and Methyl Red reactions, were performed by orthodox methods described by Mackie and McCartney (1953).

RESULTS:

During the period October, 1951, to March, 1954, inclusive, specimens of faeces or rectal swabs from a total of 5,113 patients were examined bacteriologically. Three hundred and one patients were found to be harbouring one or other of the E. coli strains under investigation.

In each of these patients the strain was examined in detail on only the first occasion of its isolation. The strains retained for detailed serological and biochemical investigations are listed in Table 1.

Table 1

Strains of E. coli investigated

Type	0111	055	026	0125	0126	086	Total
No. of strains investigated	158	42	26	60	15	0	301

Characteristics of Escherichia strains isolated:

All three hundred and one strains examined were gram-negative, aerobic, non-sporing bacilli which grew well on 2% nutrient agar at 37°C., and at room temperature.

Biochemical Reactions:

The organisms had the following biochemical reactions,

standard for the Escherichia group (Kauffmann, 1954). Except where otherwise indicated, acid and gas were produced within 24 hours in glucose, lactose, mannite, arabinose and xylose. Fermentation of other carbohydrates was variable. All strains produced indole, did not split urea, did not liquefy gelatin, did not produce H_2S, gave negative Voges-Proskauer and positive Methyl Red reactions. Citrate utilisation by some strains was noted.

E. coli O111 B4:

Details of biochemical and serological findings in respect of the one hundred and fifty-eight strains of E. coli O111 B4 are given in Table 2.

It will be seen that three serological types of E. coli O111 B4 were isolated during the survey. Types 1 and 2 could not be differentiated with accuracy on grounds of biochemical behaviour but Type 3 had a biochemical pattern quite distinct from the other types. Table 2 shows that all strains of Type 3 failed to ferment sucrose, were late fermenters of maltose and rhamnose and did not produce gas from the latter substance. These findings are in general agreement with those of Kauffmann and Dupont (1950) though no indication was given by these workers as to gas production in substances other than mannite and sucrose. It was not possible, therefore, to compare the present findings in respect of rhamnose with those of Kauffmann and Dupont. Further, in the case of sucrose, it was not uncommon to find that fermentation of this substance by

Table 2

Biochemical behaviour of representative strains of E. coli 0111 B4, with corresponding serological findings

	Fermentation Types		
	1	2	3
Sucrose	$^{1-3}_{+}$	$^{1-3}_{+}$	-
Dulcite	$^{2-5}_{+}$	$^{2-6}_{+}$	$^{4-6}_{+}$
Salicin	$^{3-11}_{+}$	$^{3-8}_{+}$	$^{3-5}_{+}$
Rhamnose	+	+	$^{3-10}_{*}$
Sorbitol	$^{1-5}_{+}$	$^{1-5}_{+}$	+
Maltose	+	+	$^{3}_{+}$
Adonitol	-	-	-
Inositol	-	-	-
Production of indole	Yes	Yes	Yes
Utilisation of citrate	No	No	No
Antigens	0111 B4 H2	0111 B4 H-	0111 B4 H12
No. of strains	36	105	17

In this and subsequent tables on fermentation reactions -

+ = acid and gas produced after 24 hours' incubation.

$^{2-5}_{+}$ = acid and gas produced after 2 to 5 days' incubation.

$^{3-10}_{*}$ = acid but no gas produced after 3 to 10 days' incubation.

Types 1 and 2 was delayed till the third day of incubation. Fermentation of dulcite, salicin and sorbitol was often delayed for periods longer than those indicated by Kauffmann and Dupont. It is noteworthy that all Type 2 strains isolated in the survey produced indole. Kauffmann and Dupont have isolated strains of O111 B4 H- which failed to do so. The close similarity of fermentation patterns of Types 1 and 2 is evident. It is conceded, however, that owing to great difficulty experienced in obtaining motile cultures of strains with these fermentation patterns, some strains classified as Type 2, i.e., O111 B4 H-, may, in fact, have been of Type 1, i.e., O111 B4 H2, having failed to show evidence of motility by my methods (see Section II of this chapter).

From time to time during the survey, one or other of the three serological types was found to predominate in the hospital. Table 3 summarises the findings for winter and summer periods.

Table 3

Occurrence of *E. coli* O111 B4 sub-types in Stobhill General Hospital

Period	Type of Wards	No. of patients positive for O111 B4		
		Type 1	Type 2	Type 3
October, 1951 - March, 1952	Paediatric	34	32	0
	Adult General	0	0	0
April - September, 1952	Paediatric	2	30	1
	Adult General	0	0	7
October, 1952 - March, 1953	Paediatric	0	28	0
	Adult General	0	0	0
April - September, 1953	Paediatric	0	3	4
	Adult General	0	0	0
October, 1953 - March, 1954	Paediatric	0	12	5
	Adult General	0	0	0
Totals		36	105	17

Thus, during the winter months of 1951-1952 the motile Type 1 strain of O111 B4 was isolated from 34 patients, while Type 2 occurred in 32. After March, 1952, however, only two patients were found excreting Type 1. Strains of Type 2 now predominated, and, except during the summer of 1953, continued to do so. Type 3 was first encountered during late May and early June, 1952, and thereafter occurred only infrequently. The clinical effects associated with the various serotypes are described in Chapter 3.

E. coli 055 B5:

Table 4 summaries the biochemical and serological findings in respect of the forty-two strains of E. coli 055 B5 subjected to detailed examination.

Table 4

Biochemical behaviour of representative strains of E. coli 055 B5, with corresponding serological findings

	Fermentation Types	
	1	2
Sucrose	+	-
Dulcite	+ [1 - 3]	+
Salicin	-	+ [2]
Rhamnose	+	+
Sorbitol	+	+
Maltose	+ [3 - 10]	+
Adonitol	-	+ [2]
Inositol	-	+ [2]
Production of indole	Yes	Yes
Utilisation of citrate	No	No
Antigens	055 B5 H6	055 B5 H7
Number of strains	39	3

Two serological types of E. coli 055 B5 were encountered. Type 1, occurring in 39 patients was motile, possessing antigen H6. The biochemical pattern associated with this serotype was fairly uniform and corresponded closely with that described by Kauffmann and Dupont (1950) and Wright and Villanueva (1953a). Kauffmann and Dupont noted that fermentation of sorbitol was variable but all the strains isolated in Stobhill General Hospital fermented this substance promptly within 24 hours. Strains of 055 B5 H6 could be recognised readily by their delayed fermentation of maltose. Acid and gas were produced by the majority of strains after approximately six days' incubation.

Organisms of biochemical Type 2 were motile and possessed the H7 antigen. This type was isolated from only three patients during the period of survey. The three strains examined were characterised by their ability to ferment salicin, adonitol, and inositol after 48 hours' incubation and by their failure to ferment sucrose. Wright and Villanueva (1953a) noted that strains of 055 B5 H7 were late fermenters of sucrose and adonitol and failed to ferment salicin. No mention of inositol fermentation was made by these workers.

Table 5 shows that the strain 055 B5 H6 occurred mainly during the winters of 1951-52 and 1953-54. Type 2 was isolated only in January and February, 1952, and did not establish itself in the hospital.

Table 5

Occurrence of *E. coli* 055 B5 sub-types in Stobhill General Hospital

Period	Type of Wards	No. of patients positive for 055 B5	
		Type 1	Type 2
October, 1951 - March, 1952	Paediatric	16†	3
April - September, 1952	Paediatric	2	0
October, 1952 - March, 1953	Paediatric	7	0
	Adult General	1	0
April - September, 1953	Paediatric	2	0
October, 1953 - March, 1954	Paediatric	11	0
Totals		39	3

†Type 1 isolated from one adult employed in paediatric ward.

E. coli 026 B6:

The strains of *E. coli* 026 B6 isolated in this survey could be divided into the three main biochemical types described by Orskov (1951), depending on their ability to produce gas in glucose and mannite and on their fermentation of dulcite and rhamnose. The results are shown in Table 6.

Orskov (1951) noted slight delay in fermentation of sucrose by his aerogenic Types 1 and 2. All aerogenic strains from patients in this hospital, however, fermented sucrose promptly.

Table 6

Biochemical behaviour of representative strains of E. coli 026 B6, with corresponding serological findings

	Fermentation Types		
	1	2	3
Glucose	+	+	*
Mannite	+	+	*
Sucrose	+	+	* 2 - 3
Dulcite	+ 1 - 3	-	-
Salicin	-	-	-
Rhamnose	+	-	-
Sorbitol	+	+	*
Maltose	+	+	*
Adonitol	-	-	-
Inositol	-	-	-
Production of indole	Yes	Yes	Yes
Utilisation of citrate	No	No	No
Antigens	026 B6 H-	026 B6 H11	026 B6 H-
Number of Strains	7	15	4

Table 7 records the occurrence of strains of E. coli 026 B6 during the survey.

Except during the winter of 1953 cases arose from time to time either sporadically or in very small localised epidemics such as that involving Type 3. This type was isolated from four patients in a paediatric ward during early June, 1953. It was never encountered in the hospital before or after that date.

Table 7

Occurrence of E. coli 026 B6 sub-types in Stobhill General Hospital

Period	Type of Wards	No. of patients positive for 026 B6		
		Type 1	Type 2	Type 3
October, 1951 - March, 1952	Paediatric	0	2	0
	Adult General	2	1	0
April - September, 1952	Paediatric	1	4	0
	Adult General	1	5	0
October, 1952 - March, 1953	Paediatric	0	0	0
	Adult General	0	0	0
April - September, 1953	Paediatric	0	1	4
	Adult General	0	0	0
October, 1953, - March, 1954	Paediatric	3	0	0
	Adult General	0	2	0
Totals		7	15	4

E. coli 0125 B15:

All the sixty representative strains of E. coli 0125 B15 investigated were non-motile and of uniform serological type.

Biochemical variations are shown in Table 8. These were

limited to minor variations in the fermentation of salicin and rhamnose and, in one strain, utilisation of citrate. Charter and Taylor (1952) have described three main fermentation types of 0125 B15, one of which was motile and was shown later to possess H antigen 19 (Taylor and Charter, 1955b). The biochemical reactions of the majority of non-motile strains in this investigation corresponded closely to those of one of Charter and Taylor's non-motile fermentation types.

Table 8

Biochemical behaviour of representative strains of *E. coli* 0125 B15, with corresponding serological findings

	Fermentation Types				
	1	2	3	4	5
Sucrose	+	+	+	+	+
Dulcite	+2-4	+4	+4	+2	+3
Salicin	-	-	-	+	*
Rhamnose	+2-5	*4	-	+	-
Sorbitol	+	+	+	+	+
Maltose	+	+	+	+	+
Adonitol	-	-	-	-	-
Inositol	-	-	-	-	-
Production of indole	Yes	Yes	Yes	Yes	Yes
Utilisation of citrate	No	No	No	No	Yes
Antigens	0125 B15 H-	0125 B15 H-	0125 B15 H-	0125 B15 H-	0125 B15 H-
Number of strains	51	4	3	1	1

E. coli 0125 B15 was isolated during each summer and winter period from March, 1952, to March, 1954. Of the sixty cases found

harbouring the organism, fourteen occurred during the summer months of 1952, twenty-nine during the winter of 1952-53, twelve during the summer of 1953 and five during the winter of 1953-54. The organism was isolated from paediatric wards only.

E. coli 0126 B16:

The fifteen strains of E. coli 0126 B16 investigated were of uniform biochemical type similar to that described by Charter and Taylor (1952). All were motile and possessed H antigen 2. The biochemical pattern of the strains of 0126 B16 H2 isolated in the survey is shown in Table 9. These strains differed from those of Charter and Taylor mainly in their ability to ferment sucrose promptly. With most of Charter and Taylor's strains fermentation of sucrose was delayed till the third day of incubation.

Table 9

Biochemical behaviour of representative strains of E. coli 0126 B16, with corresponding serological findings

	Fermentation Type
Sucrose	+
Dulcite	+ 2 - 4
Salicin	+ 4
Rhamnose	+
Sorbitol	+
Maltose	+
Adonitol	-
Inositol	-
Production of indole	Yes
Utilisation of citrate	No
Antigens	0126 B16 H2
Number of strains	15

This serotype was isolated from patients in the hospital mainly during the summer months of 1952 and 1953. The organism occurred both in paediatric and adult general wards. It did not, however, establish itself for any lengthy periods in these wards and its occurrence was, therefore, sporadic rather than epidemic.

DISCUSSION:

The serological classification of the Escherichia group by somatic, surface and flagellar antigens has enabled coliform organisms to be classified accurately on an antigenic basis. Felix (1952) has disputed the existence of the surface or capsular antigens, L, A and B, of Kauffmann. According to Felix these antigens are heat labile somatic antigens analogous to typhoid Vi antigen. This is an academic argument, however, which does not affect routine typing methods or the epidemiological investigation of coliform strains in infantile gastro-enteritis.

Culture of E. coli strains from stool samples or rectal swabs presents no difficulties. The search for specific serological types of E. coli in faecal cultures and the accurate serological typing of E. coli strains does, however, involve much painstaking and accurate preparatory work in the production of potent antisera and patient, often tedious, investigation of culture plates. Although cases of infantile gastro-enteritis encountered in this survey frequently yielded profuse growths of one or other E. coli serotype, in many stool samples from both acute cases and asymptomatic carriers typeable

and untypeable strains of E. coli were found present together. Often in such cases positive slide agglutination of the sample taken from the inoculation "well" indicated the presence of a specific serological type, individual colonies of which could only be demonstrated after prolonged search over the remainder of the culture plate.

Non-specific agglutination of colonies on slide testing presented difficulties, especially in the early stages of the survey, though acriflavine testing did eliminate many such spuriously agglutinating strains. As experience was gained in interpreting slide agglutination results it was found that strains could be typed in this way with a high degree of accuracy, though, of course, tube-agglutination tests were always carried out in confirmation. The serotype 0126 B16 was that most likely to be mis-reported on the results of slide agglutination. It was found that antisera prepared to this serotype tended to give positive slide agglutination readily during the screening of primary faeces cultures. Many strains provisionally reported as E. coli 0126 B16 failed to give a positive acriflavine test or a subsequently positive tube-agglutination test. It was found that many organisms giving non-specific agglutinations did so only when slide agglutinations were performed from media containing bile-salts, e.g., Sodium taurocholate or Sodium desoxy-cholate. This applied not only to E. coli 0126 B16 antisera but also to the other antisera used in the survey, and was regarded as a serious disadvantage in the use of MacConkey agar for the primary

isolation of E. coli test strains from faeces. This problem and the measures taken to overcome it were more fully investigated after completion of the survey. Details of this work are given in Section III of this chapter.

Study of the biochemical reactions of different OB serotypes shows that, as other workers have found, distinctive fermentation patterns often accompany antigenic variation in respect of flagellar antigens. An important exception is present in the case of certain strains of E. coli O111 B4. Biochemically, Types 1 and 2 of this organism are essentially similar in all respects, yet Type 1 is non-motile and Type 2 possesses H antigen 2. Type 3, i.e., E. coli O111 B4 H12, can, however, be readily differentiated from the other two types on the basis of fermentation pattern. The same is true for the various H antigenic sub-types of E. coli 055 B5 and 026 B6. It is often possible, therefore, to subtype provisionally strains of E. coli on the basis of biochemical patterns. Final classification must, however, depend on serological investigation of motile forms.

II. METHODS FOR PRODUCING FLAGELLATED FORMS OF E. COLI

The flagellar antigens of strains of E. coli isolated in the survey could be readily classified serologically, provided highly motile cultures were obtainable. It was, however, exceptional to find motile forms on first isolation and the production of motility was difficult, tedious and sometimes unsuccessful. This is a common experience and has been commented on by Vahlne (1945), Hilton and Taylor (1951), Charter and Taylor (1952), Smith (1953), Wright and Villanueva (1953b) and Kauffmann (1954). Vahlne (1945) induced motility in his cultures by inoculating them into one arm of a U-tube containing 0.1% agar, incubating at 37°C., and harvesting the growth when it had reached the surface in the second arm. Kauffmann (1954) endorses this method. The method, however, is open to objection in that incubation at 37°C. does not always yield motile cultures; indeed, it has been exceptional in the present investigation to obtain motile cultures of E. coli by incubation at 37°C. Also, for routine use and when large numbers of cultures are being dealt with, the U-tubes, by virtue of their shape and instability, are unsatisfactory to handle and store. Moreover, when incubation of a culture is prolonged, as is frequently necessary, it is difficult to be sure whether the harvested growth has progressed through the medium by induced motility or whether it has reached the second arm

by direct extension between the surface of the medium and the glass surface of the U-tube, in which case no great stimulus to flagellar formation is to be expected. This objection is also applicable to Craigie tubes when prolonged incubation is required.

Hilton and Taylor (1951) found that by serial passages of cultures through either semi-solid agar or nutrient broth and incubating at 22°C. highly motile cultures could be obtained. The period of passage necessary was three to four weeks in the case of semi-solid agar and slightly longer in broth. This technique was successfully employed by these authors with five strains of E. coli 0111 B4.

Charter and Taylor (1952), using overnight broth cultures at 22°C. for preparation of H forms, investigated a large number of coliform strains isolated from cases of gastro-enteritis in the London area. That their methods of inducing motility were imperfect is evident from their statement, viz., "It has not been possible to identify the H antigen in all strains owing to the difficulty in obtaining flagellated cultures."

Smith (1953) referred to the Charter and Taylor (1952) technique and confirmed that it was not successful in producing motility in every case.

Three further modifications of the above methods were described by Wright and Villanueva (1953b) and used with strains of E. coli 0111 B4 and 055 B5. Semi-solid agar in Craigie tubes and in bijou-bottles, and MacConkey agar also in bijou-bottles, were

compared for production of motility. Incubation was carried out at room temperature. Investigations showed that the MacConkey agar, inoculated with a straight wire as a "stab" culture, induced satisfactory motility when growth had advanced up the "stab" line and over the free surface of the medium. This was the method of choice and so confident were the authors in its efficiency that ultimately, for H antigen determination, motilities were not tested and growth was harvested directly from the surface of the medium by washing off in 0.25% formal-saline.

Trial of MacConkey agar has failed to confirm Wright and Villanueva's experience. One of the difficulties arising in the use of the method has been determining when surface growth is present, since the thin layer of uniform growth can be very difficult to see in a bijou-bottle. In cases where visible growth occurred over the surface, addition of N/1 saline or nutrient broth, followed by microscopic examination showed that motility was not present in every case.

Having tested out the above methods and experienced great difficulty in producing motile forms, it was found that a modified Gard swarm-agar technique (Kauffmann, 1951,) gave better results than any other method.

MATERIALS AND METHODS:

0.2% nutrient agar was poured into 4" petri plates and allowed to cool. The centre of each plate was then lightly inoculated with a broth culture of the organism under test. Incub-

ation was carried out on the bench at room temperature (approximately 20°C.) for up to 72 hours. Plates were examined daily.

After 24 hours' incubation growth was seen as a central circumscribed white patch. The development of motility was indicated by a fine film of growth seen as a greyish turbidity spreading from the central white area to cover all or most of the surface of the plate. A subculture was made from the outer area of the growth into nutrient broth which was again incubated at bench temperature for 18 hours. The broth culture was examined microscopically and if over 80% of the organisms showed active motility commercial formalin was added to the broth to make a final concentration of 0.25%. The formolised culture was then used as antigen in tube agglutination tests with H antisera, in order to determine the flagellar type of the organism.

In many cases satisfactory spread of growth did not take place within the first 72 hours. In such cases, and also where motility was present in fewer than 80% of organisms examined, subcultures from the growing edge were made to a second 0.2% swarm-agar plate. The plate was then re-incubated for 72 hours and examined as before. If still non-motile, or only sluggishly motile, subcultures were made for a third time and again incubated for up to 72 hours. It was found that after a third subculture all organisms showing any degree of motility on earlier cultures had become actively motile and were satisfactory for H antigen determination. Any strain which failed to show characteristic spread beyond the

central area of primary growth after subculturing three times was designated a non-motile strain.

The method described was compared with Craigie tube and MacConkey stab culture methods for production of motile forms of E. coli. Twelve strains of E. coli 0111 B4 and twelve of 055 B5 were used as test organisms. Incubation was carried out at bench temperature for a period of up to 72 hours. Growths from Gard plates and Craigie tubes were then sub-cultured in nutrient broth for a further 18 hours at room temperature and examined for motility using a hollow-ground slide and cover-slip. Growths from the surface of MacConkey stab cultures were suspended in nutrient broth, as described by Wright and Villanueva (1953b), and these suspensions were then examined for motility.

RESULTS:

Table 10 summaries the results obtained.

Table 10

Comparison of methods for inducing motility in cultures of E. coli

Method	No. of strains examined	No. of strains showing motility	No. of strains remaining non-motile
Gard swarm-agar plate	24	15	9
Craigie tube		7**	17*
MacConkey stab		8**	16

*These strains failed to reach outer surface of medium after 72 hours' incubation at bench temperature.

**These motile cultures also showed motility by Gard swarm-agar plate method.

The results show that the Gard plate technique was more successful for production of motility than were the Craigie tube and MacConkey stab methods. In the strains examined neither of the latter methods induced motility which was not demonstrable by the Gard plate method.

DISCUSSION:

For successful demonstration of the H antigens of E. coli it is important to obtain motile flagellated cultures. Existing methods for producing motile forms of E. coli have been described and objections to these methods noted.

The Gard plate method was adopted in this survey since it appeared to be more efficient in producing motility than were other recognised methods. Evidence of motility was easily discernable by growth characteristics on the surface of the Gard plate and this enabled non-motile strains to be readily recognised without recourse to subculture in broth and subsequent microscopic examination. Moreover, by virtue of the simplicity of materials and procedures employed, the method lent itself to use in the routine investigation of large numbers of E. coli strains.

III. SELECTIVE ISOLATION OF SPECIFIC E. COLI SEROTYPES FROM FAECES

The isolation of E. coli serotypes from stool samples is a crude and time-consuming procedure. The method used during the hospital survey was on the orthodox lines already described in Section I of this chapter. This method was found to have two serious disadvantages.

1. Specific serological types of E. coli could not be differentiated by colonial appearances from other lactose fermenting organisms normally present in stool cultures. Isolation of test types was, therefore, largely a matter of chance.

2. The occurrence of false positive agglutinations on slide testing of MacConkey cultures led to difficulty in interpreting results, especially during the earlier part of the survey when experience was minimal. Preliminary investigation of this phenomenon indicated that its occurrence was often related to the presence of bile salts in the culture medium.

These disadvantages illustrate the need for more efficient and selective methods of isolation. One great difficulty besetting all efforts to produce a selective medium for culture of strains of E. coli associated with gastro-enteritis is that, to date, no completely specific cultural or biochemical differences have been shown to exist between these strains and "normal" strains of E. coli. Rappaport and Henig (1952) used the late or non-fermentation of sorbitol as a means of differentiating strains of E. coli 0111 B4 and 055 B5 from other coliform strains in primary plate cultures.

Study of sorbitol fermentation by the strains of E. coli 0111 B4, 055 B5, 026 B6, 0125 B15 and 0126 B16 isolated in this survey shows that, for the most part, these strains fermented sorbitol promptly within 24 hours. Moreover many strains of Enterobacteriaceae other than Escherichia types are known to ferment this carbohydrate promptly (see Table 11), so that differentiation of E. coli strains on the basis of sorbitol fermentation is not really of much practical value.

The results of biochemical investigations on organisms isolated in the survey indicated that fermentation of the glucoside salicin by strains of E. coli 0111 B4, 055 B5, 026 B6, 0125 B15 or 0126 B16 was either absent or delayed beyond the first 24 hours of incubation. Further, it is evident from Smith's summary of the biochemical patterns of other strains of E. coli associated with gastro-enteritis that, with the exception of serotypes 0119 B14 and 0128 B12, none of these organisms ferments salicin promptly (Smith, 1955). Personal experience with recently isolated strains of 0119 B14 or 0128 B12 has shown, however, that while these strains ferment salicin they do not as a rule do so within the first 24 hours of incubation. It was, therefore, reasoned that incorporation of salicin and an indicator in a culture medium would be helpful in differentiating potentially enteropathogenic types of E. coli from salicin fermenting E. coli strains and also from other salicin fermenting Enterobacteriaceae liable to be met with in stool cultures (see Table 11).

Table 11

Sorbitol and salicin fermentation reactions of Enterobacteriaceae (derived from Kauffmann, 1954)

	Fermentation Reactions	
	Sorbitol	Salicin
Escherichia	+	x
Salmonella	+	-
Arizona	+	-
Bethesda-Ballerup	+	late or -
Klebsiella	+	+
Cloaca	-	+
Haffnia	-	x
Shigella	x	-
Proteus	-	+ or x
Providencia	-	-

\+ = fermentation
\- = no fermentation
x = fermentation variable.

In the preparation of the culture medium bile salts were excluded for reasons already given. It was, therefore, necessary to take steps to prevent swarming of Proteus strains and this was accomplished by using agar in a concentration of 6%. After trial of several formulae, the following one was found to give satisfactory

and reproducible results:-

Culture medium for primary isolation of *E. coli* serotypes from faeces

Nutrient broth (as described by Mackie and McCartney, 1953)	1,000 ml.
Sodium dihydrogen phosphate	1 gram.
Disodium hydrogen phosphate	1 gram.
Salicin	10 grams.
Bromthymol blue solution	15 ml.
Powdered agar (Difco Ltd.)	60 grams.

Bromthymol blue solution

Bromthymol blue powder		0.4 gram.
N/10 Sodium hydroxide		6.4 ml.
Distilled water	to	100 ml.

Preparation of medium:

The sodium and disodium phosphates are dissolved in 1,000 ml. of nutrient broth. Vigorous shaking facilitates solution. The pH of the solution is adjusted to 6.8 using N/10 hydrochloric acid. Powdered agar is then added and dissolved by steaming at 100°C. Filtration should not be necessary. At this stage the medium is bottled in 200 ml. amounts and sterilised by autoclaving for thirty minutes at a pressure of 15 lb./sq. in.

For use, this basic medium is melted. 2 grams of salicin and 3 ml. of the bromthymol blue solution are added to each 200 ml.

amount and mixed well. The medium is poured into 4" petri dishes, allowed to set, then thoroughly dried in an incubator.

The final product is slightly opalescent and of a bluish-green colour. Since the medium is buffered, it can be stored at room temperature for several days before use without any change of pH being evident.

For convenience of description the medium is referred to as S.B.A. (Salicin-Bromthymol Blue-Agar) Medium.

Growth characteristics of various species on S.B.A. medium:

Salicin fermenting organisms appear as bright yellow colonies; non-salicin fermenters are blue. Strains of E. coli grow readily on the medium and colonies reach maximum size of approximately 3 mm. diameter after 18 to 24 hours' incubation at 37°C. Colonies of E. coli are convex with raised opaque centres and wavy edges. The non-salicin-fermenting strains of E. coli cannot be distinguished from members of the Salmonella or Shigella groups. Proteus strains may or may not ferment salicin. In general, Proteus colonies have a somewhat more mucoid appearance than those of E. coli, though great experience would be required to distinguish non-salicin-fermenting Proteus strains from non-salicin-fermenting strains of E. coli on grounds of colonial appearances. Klebsiella strains all ferment salicin. Klebsiella colonies are small and do not have the mucoid appearances so characteristic of the species when grown on nutrient agar or MacConkey agar.

Comparison of S.B.A. medium and MacConkey medium for the primary isolation of E. coli serotypes from stool cultures:

This investigation was carried out in Ruchill Hospital, Glasgow, on faeces specimens and rectal swabs received from babies under eighteen months old. Each specimen was inoculated directly on to S.B.A. and MacConkey culture plates. Cultures were examined after eighteen to twenty-four hours' incubation at 37°C. Screening of the replicate cultures by slide agglutination was carried out as already described (p. 25). For purposes of the investigation two pooled antisera covering E. coli types O111 B4, O55 B5, O26 B6, O86 B7, O125 B15, O126 B16, O25, O119 B14, O127 B8 and O128 B12 were used. Serological examination of individual colonies was confined to those fermenting lactose in the case of MacConkey medium and to those failing to ferment salicin in the case of S.B.A. medium. Tube agglutination tests were performed to confirm slide agglutination results.

RESULTS:

Four hundred specimens of faeces or rectal swabs were cultured in parallel on the two media. The results were compared and are summarised in Table 12.

The results taken overall show that S.B.A. medium allowed 73 (18.25%) of the 400 cultures to be discarded without recourse to slide agglutination, on the grounds of salicin fermentation by all colonies. One hundred and eighty-two cultures (45.5%) contained salicin-fermenting and non-salicin-fermenting colonies. S.B.A.

Table 12

Comparison of growths obtained on MacConkey and S.B.A. media in 400 specimens

MacConkey Culture Plates	S.B.A. Culture Plates			Totals
	Number all salicin fermenters	Number all non-salicin fermenters	Number mixed salicin fermenters and non-salicin fermenters	
Number all lactose-fermenters	67	128	152	347
Number all non-lactose fermenters	2	3	7	12
Number mixed lactose fermenters and non-lactose fermenters	4	14	23	41
Totals	73	145	182	400

medium was selective in these 182 instances to the extent that only non-salicin-fermenting colonies required serological screening. In the remaining 145 cultures (36.25%), S.B.A. medium was not selective in any way since these cultures contained colonies, all of which failed to ferment salicin.

With MacConkey medium, on the other hand, only 12 culture plates (3%) could be discarded because of non-fermentation of lactose by all colonies. In 41 others (10%) selection of lactose-fermenting from non-lactose fermenting colonies was possible. In the remaining 347 plates (87%) which showed uniform fermentation of lactose by all colonies, selection of colonies for screening was a

matter of chance only. It is noteworthy that of the 347 corresponding S.B.A. cultures, 67 did not require serological examination and in another 152, selective screening of colonies was possible.

A total of 92 specific serological types of E. coli were isolated during the investigation. These are listed in Table 13.

Table 13

Serological types of E. coli strains isolated

Serological Type	0111 B4	055 B5	026 B6	0125 B15	0126 B16	0127 B8	0128 B12	0119 B14	086 B7
No. of strains isolated	9	34	14	25	0	1	4	2	3
Total	92								

Table 14 records the isolation of specific serotypes obtained on each of the two culture media.

The serotypes being sought were isolated on S.B.A. medium in fifteen instances in which MacConkey medium gave negative results. The converse was obtained in only one specimen.

Table 14

The isolation of specific E. coli serotypes on S.B.A. and MacConkey Media

Total No. of serotypes isolated	No. isolated on both media	No. isolated on S.B.A. medium only	No. isolated on MacConkey medium only
92	76	15	1

During the investigation non-specific agglutination of organisms on slide testing led to difficulties in only a few instances and adequate comparison of the two media in this respect was not possible.

DISCUSSION:

It is apparent from study of cultural and biochemical characteristics of the E. coli serotypes associated with gastro-enteritis that these types do not differ from many "normal" strains of E. coli. Consequently, production of a satisfactory selective medium for the isolation of potentially enteropathogenic strains has proved difficult. Comparison of the salicin-containing S.B.A. medium with standard MacConkey medium in the present investigation showed that, while S.B.A. medium was not specifically selective for "gastro-enteritis types" of E. coli, it did to a greater extent than MacConkey medium indicate which colonies were worthy of sero-logical screening. In this way the element of chance, so prevalent in examination of MacConkey cultures was reduced and this is held to account for the greater number of positive isolations obtained with S.B.A. medium. For the same reason, time spent in investigating primary stool cultures was less with S.B.A. medium than with MacConkey because S.B.A. culture plates allowed 73 (18.25%) of 400 cultures to be discarded on colonial appearances alone, whereas this was possible with only 12 (3%) of the 400 corresponding MacConkey cultures.

It is considered that the greater efficiency of S.B.A. medium compared with MacConkey medium in these respects and also its ease of preparation make this medium a suitable one for use in the routine culture of stools for potentially enteropathogenic *E. coli*.

CHAPTER 3

PATHOGENICITY OF ESCHERICHIA COLI

I.

THE CLINICAL EFFECTS ASSOCIATED WITH SPECIFIC SEROLOGICAL TYPES OF E. COLI

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THE CLINICAL EFFECTS ASSOCIATED WITH SPECIFIC SEROLOGICAL TYPES OF E. COLI

Over the past decade several serological types of E. coli have come to be regarded as enteropathogenic agents largely as the result of clinical and bacteriological observations made on babies in paediatric units or nurseries. Information is lacking on the occurrence of these strains in general hospitals.

The present chapter deals with the incidence and enteropathogenic effects of specific serological types of E. coli in patients of all ages in Stobhill General Hospital, Glasgow.

The hospital serves an urban population of approximately 250,000 and has 1,200 beds dealing with general medicine and surgery, otorhinolaryngology, ophthalmology, paediatrics, obstetrics and gynaecology, dermatology, tuberculosis and psychiatry.

The investigation was carried out over a period of thirty months from October, 1951, to March, 1954. During this period all faecal specimens and rectal swabs received from the wards were examined for specific serological types of E. coli and for members of the Salmonella and Shigella groups of intestinal pathogens, as described in Chapter 2.

The selection of specimens for bacteriological examination

was made primarily by clinicians on clinical grounds, i.e., when symptoms were present or where the physician or surgeon required bacteriological information.

RESULTS:

Specimens were received and examined from a total of 5,113 patients. The positive bacteriological findings are summarised in Table 15. All specimens were examined for E. coli serotypes 0111 B4, 055 B5 and 026 B6 and for Shigella and Salmonella strains. The 3,805 specimens submitted after March, 1952, were also examined for E. coli 0125 B15, 0126 B16 and 086 B7.

Table 15

Occurrence of specific E. coli serotypes and of members of Salmonella and Shigella Groups in Stobhill General Hospital, October, 1951, to March, 1954

Period	Total No. of patients examined	No. of patients harbouring specific E. coli serotypes						No. of patients harbouring other pathogens	
		0111 B4	055 B5	026 B6	0125 B15	0126 B16	086 B7	Shigella	Salmonella
October, 1951, - March, 1952	1,308	66	19	5	-	-	-	27	4
April, 1952, - March, 1954	3,805	92	23	21	60	15	0	66	11
Totals	5,113	158	42	26	60	15	0	93	15
Incidence %	-	3.1	0.8	0.5	1.6	0.4	0	1.8	0.3

These results show that the total incidence of E. coli serotypes among the patients examined was greater than that of either Shigella or Salmonella strains. A high proportion of specimens, however, came from babies under 1 year old and from children between the ages of 1 and 12 years. Table 16 shows the numbers examined and the incidence of E. coli, Salmonella and Shigella strains in each age group.

Table 16

Number of patients examined in various age groups with incidence per cent. of E. coli, Salmonella and Shigella Strains

Strains sought	Incidence % in each age group and number of patients examined					
	Adult		1 to 12 years old		Under 1 year old	
Shigella	2.4		3.4		0.5	
Salmonella	0.5		0.4		0.05	
0111 B4	0.3	1,911	1.2	1,084	6.6	2,118
055 B5	0.1		0.4		1.7	
026 B6	0.5		0.1		0.7	
0125 B15	0		0.9		3.4	
		1,475		771		1,559
0126 B16	0.2		0.3		0.6	

It is evident from Table 16 that the relatively high incidence of E. coli serotypes compared with organisms of Salmonella and Shigella groups was due to the frequent occurrence of these serotypes in babies less than one year old. In the older age groups the incidence of E. coli serotypes was greater than that of Salmonella

strains and less than that of Shigella strains.

Table 17 shows that the strains of E. coli under investigation were isolated at all times of the year though rather more often in winter and early spring months. The sexes were found to harbour these organisms with equal frequency.

Table 17

Occurrence of specific E. coli serotypes month by month

Year	No. of patients harbouring E. coli serotypes in												Totals
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
1951	-	-	-	-	-	-	-	-	-	19	11	19	49
1952	13	9	19	9	14	17	9	13	11	11	10	9	144
1953	16	8	12	2	6	7	5	5	7	7	3	9	87
1954	10	8	3	-	-	-	-	-	-	-	-	-	21
Total													301

Clinical effects of E. coli serotypes:

The symptoms and signs of gastro-enteritis to some extent vary according to whether the patient is an infant, a toddler, a school-child or an adult. The one sign common to all age groups is diarrhoea, which gives rise in turn to a greater or lesser degree of dehydration.

Accordingly pathogenic effects of the specific serological types of E. coli isolated in this survey were judged by the

presence and mutual relationship of diarrhoea and dehydration in cases excreting these types. Diarrhoea was taken to mean any increase in frequency or alteration in consistency of stools. Diarrhoea was said to be severe when resulting dehydration was sufficient to cause the administration of parenteral fluids. All other diarrhoea was regarded as mild.

Patients harbouring E. coli 0111 B4:

The serological type 0111 B4 was that most frequently isolated in the survey. This organism was found in the faeces of one hundred and fifty-eight patients. Of these patients, fifty-one (32.3%) had severe diarrhoea, sixty-four (40.5%) had mild diarrhoea, and forty-three (27.2%) were without gastro-intestinal symptoms. Three cases died, a mortality rate of 1.9%.

Table 18 shows the distribution of cases according to age-groups, the degree of concomitant diarrhoea, and whether the organism was present on admission or contracted during the patient's stay in hospital.

It is evident from the last column of the table that under the age of 1 year there was a fairly uniform incidence of E. coli 0111 B4 among the patients examined. Over the age of 1 year it was comparatively rare to isolate the organism.

Patients infected outside hospital:

Forty-five patients were admitted to hospital excreting E. coli 0111 B4. All of these patients were under 18 months old and were admitted as cases of "dysentery" or "gastro-enteritis".

Table 18

The occurrence of gastro-enteritis in association with E. coli 0111 B4

Age Group	Total No. of patients examined	0111 B4 first isolated from faeces						Total Isolations	Incidence of E. coli 0111 B4 in cases examined
		On admission			After admission				
		Degree of diarrhoea			Degree of diarrhoea				
		Mild	Severe	Absent	Mild	Severe	Absent		
Under 1 month old. Born in hospital	445	-	-	-	12	1	12	25*	5.6%
Premature	167	-	-	-	3	2	1	6	3.6%
Under 3 months	788	11	10	2	14	13(2)	12	62	7.9%
4 to 12 months	718	6	10	3	11	11(1)	6	47	6.5%
1 to 12 years	1,084	-	3	-	5	-	5	13	1.2%
Adult	1,911	-	-	-	2	1	2	5	0.3%
Totals	5,113	17	23	5	47	28(3)	38	158	3.1%

*10 of these infants were premature.

Figures in brackets represent deaths.

Only five patients (11.1%) had no symptoms after admission, while twenty-three (51.1%) had severe diarrhoea and seventeen (37.8%) mild diarrhoea.

Patients infected after admission:

One hundred and thirteen patients became infected with E. coli O111 B4 while in hospital. In contrast to patients positive on admission, thirty-eight of the hospital patients (33.6%) had no symptoms referable to the presence of O111 B4 in their faeces. Twenty-eight (24.8%) had severe diarrhoea and forty-seven (41.6%) mild diarrhoea.

Severe cases occurred most frequently among infants. In babies under 1 year, other than those born in hospital, there was a consistent relationship between the presence of E. coli O111 B4 and the occurrence of severe diarrhoea. Thus two (33.3%) of six premature babies, thirteen (33.3%) of thirty-nine infants up to 3 months old and eleven (39.3%) of twenty-eight babies between the ages of 4 and 12 months had severe diarrhoea following infection in hospital with E. coli O111 B4. Of the babies born in hospital, however, only one (4%) of twenty-five had severe diarrhoea. Babies born in hospital and those under one year old admitted to hospital were being nursed in similar environmental conditions but only the former group was receiving or had recently received breast milk. This possibly accounts for the smaller number of severely ill cases occurring among infants born in the hospital since it is recognised that gastro-enteritis is rare in breast fed infants (Smith, 1955).

The results shown in Table 18 indicate that patients of all age groups can be found to harbour E. coli O111 B4 and remain symptomless.

This has been commented on before, notably by Payne and Cook (1950) as casting doubts on the pathogenicity of this organism.

Since approximately one third of the patients in the present series were unaffected by the presence of E. coli O111 B4 in their gastro-intestinal tracts, factors which might be liable to predispose to the development of gastro-enteritis were noted. The factors considered were (1) debility of the patient, and (2) antigenic and biochemical type of organism isolated.

Environment in hospital was not considered as the great majority of patients were being nursed in the same type of environment, namely, open wards.

(1) Debility:

The debilitating factors investigated were:-

1. The presence of systemic disease.
2. A recent attack of gastro-enteritis.
3. A recent or concomitant parenteral infection.
4. Extremes of age, viz., prematurity or senility.

The results are summarised in Table 19.

In the adult age group, all five patients from whom E. coli O111 B4 was isolated had other systemic disease at that time. The severe case, a woman of 73 years, had auricular fibrillation and cardiac failure. One of the two mild cases was a man of 79 years who had had a left mid-thigh amputation for arterio-sclerotic gangrene two days prior to the onset of gastro-enteritis. The second mild case was a 59 year old woman who had glaucoma and severe diabetes. Of the two symptomless excretors, one was a woman of

Table 19

Clinical severity of gastro-enteritis in relation to debility

Age Group	Total positive O111 B4	Degree of Diarrhoea	Numbers affected	Debilitating Factors					Total Debilitated
				Systemic Disease	Parenteral Infection	Recent Gastro-enteritis	Prematurity	Senility	
Adult	5	Mild	2	2	0	0	-	1	5
		Severe	1	1	0	0	-	1	
		Nil	2	2	0	0	-	2	
Premature	16	Mild	5	0	0	0	5	-	16
		Severe	6	0	0	0	6	-	
		Nil	5	1	0	1	5	-	
Non-premature Under 3 months	77	Mild	35	2	6	2	-	-	24
		Severe	20	3	1	1	-	-	
		Nil	22	3	4	2	-	-	
4 to 12 months	47	Mild	17	1	7	0	-	-	28
		Severe	21	0	10	0	-	-	
		Nil	9	3	6	1	-	-	
1 to 12 years	13	Mild	5	0	4	1	-	-	10
		Severe	3	0	0	0	-	-	
		Nil	5	0	5	0	-	-	
Total	158		158	18	43	8	16	4	83

84 years suffering from anaemia and senility, and the other, a woman, aged 66, who had hypertension.

It is evident that these five patients were old and debilitated by disease, and while these factors may to some extent have predisposed to the establishment of the organism in the bowel, they would appear, per se, to have had no very clear relationship to the development of gastro-intestinal disturbance.

In the premature baby group, it is reasonable to assume that all the infants were debilitated. Reference to Table 19 shows that systemic disease, infection or previous gastro-enteritis could not account for the development of symptoms. It will be seen that the proportions of patients having mild diarrhoea, severe diarrhoea and no diarrhoea were similar to those found in the other age groups and, therefore, the state of prematurity did not appear to influence the severity of the disease.

Twenty severe cases occurred among the non-premature babies under 3 months old. In only five of these cases, however, could debilitating factors be found. Three infants had systemic disease, and the fourth was recovering from broncho-pneumonia. The fifth had had mild diarrhoea associated with E. coli 026 B6 for two days before the isolation of E. coli 0111 B4 and the onset of severe diarrhoea. Similarly, of the thirty-five babies having mild diarrhoea, only ten could be shown to be debilitated. Two of those babies had systemic disease, six had parenteral infections and two had recently recovered from gastro-enteritis in another

hospital. Again debilitating factors could not be shown to influence the severity of the disease to any great extent.

In the 4 to 12 month age group, twenty-one patients had severe diarrhoea associated with the isolation of O111 B4 from the stools. Ten of these patients had parenteral infections (5 respiratory, 2 otitis media, 1 bronchitis and otitis media, 1 infantile eczema and 1 umbilical infection). No case had other systemic disease and nonehad recently had gastro-enteritis. So that in fewer than half the babies having severe diarrhoea could debilitating factors be found. In eight of the seventeen mild cases debility was present. Seven had parenteral infections, viz., five, one a Mongol, had respiratory tract infections, another was recovering from meningococcal meningitis and the seventh had tonsillitis. The remaining mild case had systemic disease - renal acidosis. Thus, in this group, also, the presence of debilitating factors could not definitely be related to the development of symptoms.

Nine of the thirteen O111 B4 positive patients over 12 months old had parenteral infections, but no severe case occurred among those nine. Four had mild diarrhoea and five remained symptomless. One further case of mild diarrhoea was found to be excreting Sh. sonnei simultaneously. No case had systemic disease.

As in the other age groups, therefore, debilitating factors in the patient appeared to play no definite part in the development of gastro-intestinal symptoms.

(2) Severity of gastro-enteritis in relation to serological and biochemical types of E. coli 0111 B4

It has been noted in Chapter 2 that three serological types of E. coli 0111 B4 were isolated during this investigation. These were the non-motile serotype 0111 B4 H-, and the motile 0111 B4 H2 and 0111 B4 H12 types. Of the one hundred and fifty-eight patients found excreting E. coli 0111 B4, one hundred and five yielded the non-motile variant, thirty-six the H2 serotype and seventeen 0111 B4 H12. The biochemical reactions of the non-motile and H2 serotypes were similar to one another and distinct from those of the H12 variety (Chapter 2).

Isolations of E. coli 0111 B4 H- and 0111 B4 H2 were confined to four paediatric wards in the hospital. The 0111 B4 H12 serotype occurred in three of these four wards, in a children's dermatological ward and in three general wards for adult patients.

Table 20 shows the severity of gastro-enteritis associated with each serological type.

Table 20

Degrees of gastro-enteritis associated with the main variants of E. coli 0111 B4

Variant	Number of patients with positive stool cultures	Degree of associated gastro-enteritis		
		Mild	Severe	Nil
0111 B4 H- Fermentation Type 2	105	42 (40)	40 (38.1)	23 (21.9)
0111 B4 H2 Fermentation Type 1	36	15 (41.7)	9 (25)	12 (33.3)
0111 B4 H12 Fermentation Type 3	17	7 (41.2)	2 (11.8)	8 (47)
	158	64	51	43

Figures in brackets represent percentages.

Taking account of the discrepancies in the total numbers isolated, no significant differences were found in the degrees of illness associated with each variant of E. coli 0111 B4.

Patients harbouring E. coli 055 B5:

E. coli 055 B5 was isolated from a total of forty-two patients. Ten (23.8%) had severe diarrhoea, seventeen (40.5%) had mild diarrhoea and fifteen (35.7%) were without gastro-intestinal symptoms.

Table 21 shows the distribution of cases according to age groups.

The occurrence of E. coli 055 B5 was less frequent than that of 0111 B4, but like the latter organism cases occurred most frequently under the age of one year.

Patients infected outside hospital:

Eleven patients of all ages had E. coli 055 B5 isolated from admission specimens of faeces. All of those patients were admitted because of gastro-intestinal upset and Table 21 shows that all continued to have observable symptoms in hospital. Four cases (37%) were severe, seven (63%) mild. The adult patient is of particular interest. This woman of 22 years was sent to hospital by her family doctor on 26th October, 1952, as a case of acute appendicitis. She had had pain in the right iliac fossa, vomiting and some diarrhoea for 24 hours. On admission she was having very frequent watery stools and appeared toxic. A provisional diagnosis of bacillary dysentery was made and the patient was barrier nursed.

Table 21

The occurrence of gastro-enteritis in association with E. coli 055 B5

Age Group	Total No. of patients examined	055 B5 first isolated from faeces						Total Isolations	Incidence of E. coli 055 B5 in cases examined
		On admission			After admission				
		Degree of diarrhoea			Degree of diarrhoea				
		Mild	Severe	Absent	Mild	Severe	Absent		
Under 1 month old. Born in hospital	445	-	-	-	6	0	4	10	2.2%
Premature	167	0	0	0	1	0	2	3	1.8%
Under 3 months	788	2	2	0	1	3	6	14	1.8%
4 to 12 months	718	3	1	0	1	2	2	9	1.3%
1 to 12 years	1,084	2	0	0	1	0	1	4	0.4%
Adult	1,911	0	1	0	0	1	0	2	0.1%
Totals	5,113	7	4	0	10	6	15	42	0.8%

E. coli 055 B5 was isolated in apparently pure culture from an admission specimen of faeces. A rapid uneventful recovery was made within a few days. It was learnt after admission that the patient's 5 month old child was in another hospital suffering from gastro-enteritis though no bacteriological details of the type of infection could be obtained. This case is unusual in that a young, otherwise healthy adult had a short, sharp attack of severe diarrhoea associated with E. coli 055 B5 and had had recent close contact with an infant ill with gastro-enteritis.

Patients infected after admission:

Thirty-one patients had E. coli 055 B5 isolated from their faeces during their stay in hospital. Fifteen (48.4%) had no apparent symptoms referable to 055 B5, six (19.4%) had associated severe diarrhoea, ten (32.2%) had mild diarrhoea.

It will be seen from Table 21 that the numbers in each age-group affected were very small and that severe cases, with two exceptions, occurred in the under 1 year age group.

Again, with E. coli 055 B5, as with 0111 B4, babies born in hospital and receiving breast milk had only minimal gastro-intestinal upset associated with the organism. No case of severe gastro-enteritis occurred among the ten babies in this group having positive stool cultures. Six of these babies had only mild symptoms.

Predisposing Factors:

Table 22 shows the relationship of symptoms to debilitating

Table 22

Clinical severity of gastro-enteritis in relation to debility

Age Group	Total Positive 055 B5	Degree of Diarrhoea	Numbers affected	Debilitating Factors					Total Debilitated
				Systemic Disease	Parenteral Infection	Recent Gastro-enteritis	Prematurity	Senility	
Adult	2	Mild	0	0	0	0	-	0	0
		Severe	2	0	0	0	-	0	
		Nil	0	0	0	0	-	0	
Premature	3	Mild	1	0	0	0	1	-	3
		Severe	0	0	0	0	0	-	
		Nil	2	0	1	0	2	-	
Non-premature Under 3 months	24	Mild	9	0	0	0	-	-	6
		Severe	5	0	2	0	-	-	
		Nil	10	1	2	1	-	-	
4 to 12 months	9	Mild	4	0	1	0	-	-	4
		Severe	3	0	1	0	-	-	
		Nil	2	0	2	1	-	-	
1 to 12 years	4	Mild	3	0	3	0	-	-	4
		Severe	0	0	0	0	-	-	
		Nil	1	0	1	0	-	-	
Total	42		42	1	13	2	3	0	17

factors in 055 B5 positive patients.

Only two adults were found harbouring E. coli 055 B5. It will be seen that neither patient was debilitated by disease or old age. One of these patients has already been referred to. The other was a nurse, aged 20 years, who reported sick with diarrhoea on 18th October, 1951. The diarrhoea was of sudden onset and the patient was having frequent watery stools, which yielded profuse growths of E. coli 055 B5. This nurse was employed in a paediatric ward in which were cases of gastro-enteritis, none of whom, however, was known to be excreting E. coli 055 B5. Rectal swabs were taken from all the infants in the ward and E. coli 055 B5 was isolated from a male baby aged 5 months who was convalescing from 0111 B4 gastro-enteritis, but who at this point had no gastro-intestinal symptoms. The nurse made an uneventful recovery within a few days; the baby remained symptomless.

In neither of the two adult cases, therefore, could debilitating factors be found. It is interesting that these were the only two affected adults in the series who had any close association with infants.

Among the premature infants found excreting E. coli 055 B5, Table 22 shows that debilitating factors other than prematurity were present in only one patient who, in fact, remained symptomless. No severe case occurred among these premature babies and only one had symptoms at all. Again, as with 0111 B4, prematurity did not appear to affect directly the severity of diarrhoea associated with

E. coli 055 B5.

Five severe cases of diarrhoea were recorded among the twenty-four infants in the first trimester. Only two of these cases had debilitating factors demonstrable. One had thrush and a slight upper respiratory infection, the other developed gastro-enteritis while under treatment for carbuncle of kidney. None of the infants having mild diarrhoea was debilitated by previous or intercurrent disease.

In the 4 to 12 months age group, three of the nine babies had severe diarrhoea. Debilitating factors were present in only one case, however, a baby of 9 months who had intercurrent broncho-pneumonia. Similarly, among the four mild cases, there was only one who could be termed debilitated, this child also having broncho-pneumonia.

Finally among children over 1 year old, it will be seen from Table 22 that no severe case of diarrhoea occurred in association with E. coli 055 B5. All three mild cases were acutely ill with respectively, tuberculous meningitis, otitis media and bronchitis. In spite of these infections, diarrhoea did not become severe in any of the children.

It would appear, therefore, that, as with 0111 B4 cases, debility could not be directly related to the development of diarrhoea in patients harbouring E. coli 055 B5.

Severity in relation to serological and biochemical sub-types of *E. coli* 055 B5:

Two serotypes of 055 B5 occurred during the investigation. The more common, occurring in thirty-nine cases, was the motile 055 B5 H6 type. The remaining three strains were also motile and possessed H antigen 7.

Table 23 shows the association of symptoms with the two serological types present in this investigation. Biochemical variation within each serotype was minimal (Chapter 2).

Table 23

Degrees of gastro-enteritis associated with the main variants of *E. coli* 055 B5

Variant	No. of patients having positive stool cultures	Degree of Associated Gastro-enteritis		
		Mild	Severe	Nil
055 B5 H6 Fermentation type 1	39	14 (35.9)	10 (25.6)	15 (38.5)
055 B5 H7 Fermentation type 2	3	3 (100)	0	0
Total	42	17	10	15

Figures in brackets represent percentages.

There is no evidence that the severity of gastro-enteritis was directly related to one particular biochemical or serological sub-type of *E. coli* 055 B5

Patients harbouring *E. coli* 026 B6:

Twenty-six patients yielded *E. coli* 026 B6 from stool

cultures. Four (15.4%) of these patients had severe diarrhoea, thirteen (50%) had mild diarrhoea, and nine (34.6%) remained symptomless.

Table 24 shows the distribution of cases.

Ten of the twenty-six patients found harbouring E. coli 026 B6 were adults. This is a considerably higher proportion positive than was found with any other typeable strain of E. coli. There is no satisfactory explanation for this finding. It is evident that all but one of the affected adults began to excrete the organism after admission. The presumption is that these patients became cross-infected in hospital though no primary source of infection could be found. This is perhaps not altogether surprising considering the fact that all these patients were in open wards into which came a regular stream of visitors several times per week.

Patients infected before admission:

Six patients had E. coli 026 B6 isolated from admission specimens of stool. One infant (16.7%) was admitted severely dehydrated with a two day history of diarrhoea and vomiting at home. Three other patients (50%), one a woman of 52 years, had mild diarrhoea on admission and for a few days afterwards. Two babies (33.3%), with stool cultures positive for E. coli 026 B6 on admission, remained symptomless.

Patients infected after admission:

Twenty patients had E. coli 026 B6 first isolated from

Table 24

The occurrence of gastro-enteritis in association with E. coli 026 B6

Age Group	Total No. of patients examined	026 B6 first isolated from faeces						Total Isolations	Incidence of E. coli 026 B6 in cases examined
		On admission			After admission				
		Degree of diarrhoea			Degree of diarrhoea				
		Mild	Severe	Absent	Mild	Severe	Absent		
Under 1 month old. Born in hospital	445	-	-	-	1	0	2	3	0.7%
Premature	167	0	0	0	0	0	0	0	0%
Under 3 months	788	1	1	1	2	2	1	8	1.0%
4 to 12 months	718	1	0	1	1	0	1	4	0.6%
1 to 12 years	1,084	0	0	0	1	0	0	1	0.1%
Adult	1,911	1	0	0	5	1	3	10	0.5%
Totals	5,113	3	1	2	10	3	7	26	0.5%

stools after admission. Nine of these patients were adults, ten were under 1 year old, and one was aged 2 years. Severe symptoms occurred in three patients (15.0%), ten patients (50%) had mild gastro-intestinal upset and the other seven (35%) remained symptomless.

It will be seen, therefore, that almost exactly similar proportions of patients had mild, severe or no symptoms in both pre and post-admission infections. Three infants born in hospital and receiving breast milk had E. coli 026 B6 isolated from their stools. One of these infants had slightly loose stools for a few days. The other two had no symptoms.

Predisposing factors:

As with E. coli 0111 B4 and 055 B5 the association of symptoms with the presence of E. coli 026 B6 in the stools was variable. Again debilitating factors in patient and the type of the organism were investigated to determine whether they could have influenced clinical severity.

Table 25 records the debilitating factors present in patients harbouring E. coli 026 B6.

Among the ten adults one severe case of diarrhoea occurred. This was a woman of 64 years who was suffering from senile dementia and broncho-pneumonia. There had been severe progressive debility for about a year and broncho-pneumonia developed a few days before death. During the patient's terminal illness there was sudden onset of profuse diarrhoea associated with E. coli 026 B6

Table 25

Clinical severity of gastro-enteritis in relation to debility

Age Group	Total Positive 026 B6	Degree of Diarrhoea	Numbers affected	Debilitating Factors					Total Debilitated
				Systemic Disease	Parenteral Infection	Recent Gastro-enteritis	Prematurity	Senility	
Adult	10	Mild	6	3	3	0	-	2	10
		Severe	1	1	1	0	-	1	
		Nil	3	2	1	0	-	1	
Premature	0	Mild	0	0	0	0	-	0	0
		Severe	0	0	0	0	-	0	
		Nil	0	0	0	0	-	-	
Non-premature Under 3 months	11	Mild	4	1	0	1	-	-	2
		Severe	3	1	0	0	-	-	
		Nil	4	0	0	0	-	-	
4 to 12 months	4	Mild	2	0	0	0	-	-	2
		Severe	0	0	0	0	-	-	
		Nil	2	1	1	0	-	-	
1 to 12 years	1	Mild	1	0	0	0	-	-	0
		Severe	0	0	0	0	-	-	
		Nil	0	0	0	0	-	-	
Total	26		26	9	6	1	-	4	14

and she died twenty-four hours later. There is no doubt that this woman was in very poor physical condition and that the severe diarrhoea at least accelerated her death.

All six mildly ill adults were debilitated to some extent. Three had pulmonary tuberculosis and three had other systemic disease. In addition, two of the tuberculous patients were senile.

On the other hand, all three symptomless adults were also debilitated by intercurrent disease and, in addition, one was markedly senile.

It would appear, therefore, that in the adult cases excreting E. coli 026 B6 no clear relationship could be demonstrated between the presence of debility and the development of diarrhoea.

This serotype was not isolated from any premature baby during the survey. Among the non-premature babies under 3 months old, three had severe diarrhoea. One of these babies had haemolytic anaemia prior to the onset of gastro-enteritis; the other two infants had no debilitating factors demonstrable. In only one of the four mild cases was the patient debilitated. This particular child was marasmic on admission. In addition, she had had mild gastro-intestinal upset associated with E. coli 0126 B16 for three weeks prior to the first isolation of 026 B6. Co-incident with the appearance of the latter organism mild diarrhoea returned but never became severe despite the poor general condition of the infant.

Finally, Table 25 shows that in none of the children over

the age of 3 months having gastro-intestinal symptoms were debilitating factors present.

The findings in the case of E. coli 026 B6 were, therefore, similar to those obtained with the serotypes 0111 B4 and 055 B5, namely, that debilitating factors in the patient could not be directly related to development of diarrhoea.

Severity of gastro-enteritis in relation to biochemical and serological sub-types of E. coli 026 B6:

Table 26 shows that gastro-enteritis occurred in association with all three sub-types of E. coli 026 B6. There is no evidence from these results that severity of illness was related to any particular serological or biochemical sub-type.

Table 26

Degrees of gastro-enteritis associated with the main variants of E. coli 026 B6

Variant	No. of patients having positive stool cultures	Degree of associated Gastro-enteritis		
		Mild	Severe	Nil
026 B6 H- Fermentation type 1	7	3 (42.8)	2 (28.6)	2 (28.6)
026 B6 H- Fermentation type 3	4	3 (75)	0	1 (25)
026 B6 H11 Fermentation type 2	15	7 (46.7)	2 (13.3)	6 (40)
Total	26	13	4	9

Figures in brackets represent percentages.

Patients harbouring E. coli 0125 B15:

It has already been noted that E. coli 0125 B15 did not become available for investigation until March, 1952. From April,

1952, till March, 1954, sixty infants and children had the organism isolated from stool specimens or rectal swabs. Only three positive cases (5%) had severe gastro-enteritis. Fifteen patients (25%) had mild diarrhoea, and the remaining forty-two children (70%) appeared unaffected by the organism.

Table 27 indicates the distribution of cases.

The organism was most frequently isolated from babies under 1 year old. Premature babies accounted for twenty-seven of the fifty-three 0125 B15 positive patients in this age group. This high proportion of prematures was due to a small epidemic which occurred in a ward of premature babies during the months January to April, 1953, when nineteen infants were involved. It was rare to encounter the organism in patients over 1 year old and no adult cases were found. It is also evident comparing Table 27 with Tables 18, 21 and 24 that patients harbouring E. coli 0125 B15 were much less prone to develop diarrhoea than was the case with E. coli 0111 B4, 055 B5 and 026 B6.

Patients infected before admission:

Seven patients had E. coli 0125 B15 isolated from an admission specimen of faeces. Four babies (57%) had concomitant mild diarrhoea, the stools of the three others (43%) remained normal. Unlike the patients excreting E. coli 0111 B4 and 055 B5 on admission, only four (57%) of these seven 0125 B15 positive cases were admitted because of gastro-intestinal upset, and severe gastro-enteritis did not occur in any patient infected outside the hospital.

Table 27

The occurrence of gastro-enteritis in association with E. coli 0125 B15

Age Group	Total No. of patients examined	0125 B15 first isolated from faeces						Total Isolations	Incidence of E. coli 0125 B15 in cases examined
		On admission			After admission				
		Degree of diarrhoea			Degree of diarrhoea				
		Mild	Severe	Absent	Mild	Severe	Absent		
Under 1 month old. Born in hospital	266	-	-	-	0	0	4*	4	1.5%
Premature	107	0	0	0	5	2	16	23	21.5%
Under 3 months	640	4	0	2	2	1	8	17	2.7%
4 to 12 months	546	0	0	0	3	0	6	9	1.6%
1 to 12 years	771	0	0	1	1	0	5	7	0.9%
Adult	1,475	0	0	0	0	0	0	0	0%
Totals	3,305	4	0	3	11	3	39	60	1.6%

*All premature.

Patients infected after admission:

Fifty-three children became excretors of E. coli 0125 B15 during their stay in hospital. Of these positive cases, three (5.7%) were severe, eleven (20.7%) were mild, and thirty-nine (73.6%) had no associated gastro-intestinal symptoms. Infants born in hospital and receiving breast milk showed no clinical effects from the presence of E. coli 0125 B15. This is in keeping with the findings in relation to serotypes 0111 B4, 055 B5 and 026 B6. In general, gastro-intestinal symptoms associated with E. coli 0125 B15 were very mild, though one of the severely ill premature babies died within three days of the onset of diarrhoea.

Predisposing factors:

Account was again taken of debilitating factors and their relation to the development of diarrhoea. The results are shown in Table 28.

It is evident that, in premature infants, debilitating factors other than prematurity were usually absent. The severe case, which was the only fatality in relation to E. coli 0125 B15, did, however, at post-mortem, have extensive pulmonary atelectasis and was, therefore, in very poor physical condition. One other premature infant had severe monilial infection of the mouth and pharynx. This infant did not, however, develop diarrhoea.

Since only seven of the twenty-seven premature cases harbouring E. coli 0125 B15 had concomitant diarrhoea, and in view of the paucity of other debilitating factors present, prematurity

Table 28

Clinical severity of gastro-enteritis in relation to debility

Age Group	Total Positive 0125 B15	Degree of Diarrhoea	Numbers affected	Debilitating Factors				Total Debilitated
				Systemic Disease	Parenteral Infection	Recent Gastro-enteritis	Prematurity	
Premature	27	Mild	5	0	0	0	5	27
		Severe	2	1	0	0	2	
		Nil	20	0	1	0	20	
Non-Premature Under 3 months	17	Mild	6	0	2	0	-	13
		Severe	1	0	0	1	-	
		Nil	10	2	6	2	-	
4 to 12 months	9	Mild	3	2	2	0	-	9
		Severe	0	0	0	0	-	
		Nil	6	1	5	1	-	
1 to 12 years	7	Mild	1	1	0	0	-	6
		Severe	0	0	0	0	-	
		Nil	6	1	3	1	-	
Total	60		60	8	19	5	27	55

No adults involved. Therefore senility not applicable.

per se appeared to have no definite influence on the development of symptoms.

Only one case of severe gastro-enteritis occurred in non-premature babies in the first trimester. The affected infant was convalescing from severe gastro-enteritis associated with E. coli 026 B6 and was very debilitated. Co-incident with the appearance of E. coli 0125 B15 in the stools, severe diarrhoea returned, the infant became dehydrated and his general condition rapidly deteriorated. These severe symptoms were present for six days, after which a gradual and complete recovery was made. In only two of the six mild cases were debilitating factors present. One baby had a concomitant upper respiratory infection, and the other developed diarrhoea during treatment for staphylococcal impetigo.

All ten infants on whom E. coli 0125 B15 had no apparent effect were ill with other conditions. Five had upper respiratory infections, one had broncho-pneumonia, two were convalescing from previous gastro-enteritis, one had a skin condition, and one was marasmic due to imperforate anus and recto-urethral fistula. Despite the presence of these conditions no gastro-intestinal upset occurred in any of these infants following isolation of E. coli 0125 B15.

Table 28 shows that no severe diarrhoea occurred in the 4 to 12 month age group. Three babies had mild diarrhoea. One was convalescing from meningococcal meningitis; another developed diarrhoea for a few days during the course of acute leukaemia. The

third child was suffering from seborrheic eczema and otitis media.

Six babies were symptomless excretors of E. coli 0125 B15. All six were debilitated to some extent by other conditions. Infections of the respiratory tract were present in four of these infants, one of whom was, in addition, recovering from gastro-enteritis associated with E. coli 026 B6. A fifth baby had pyelonephritis and the sixth a sub-dural haematoma. There was thus no constant relationship between debility and the presence of diarrhoea.

Of the seven children over 1 year old harbouring E. coli 0125 B15, only one had mild diarrhoea. Six of the seven were, however, debilitated by other illness which indicates again that debility could not be related to development of diarrhoea.

Severity of gastro-enteritis in relation to biochemical and serological sub-types of E. coli 0125 B15:

All strains of E. coli 0125 B15 isolated in this survey were non-motile and lacked a flagellar antigen. Biochemical variations in the organisms encountered were minimal (Chapter 2). Fifty-one strains were of uniform biochemical type. Minor variations in the fermentation of salicin and rhamnose and the utilisation of citrate occurred in the remaining nine strains. These differences could not be related to severity of disease.

Patients harbouring E. coli 0126 B16:

During the period March, 1952, to March, 1954, fifteen patients had E. coli 0126 B16 isolated from their stools. No

severe diarrhoea occurred in association with this organism. Six patients (40%) had mild diarrhoea; the remaining nine patients (60%) had no gastro-intestinal upset at all.

Table 29 shows the distribution of cases.

It will be seen that no case had E. coli 0126 B16 isolated from an admission specimen of stool. Seven of the ten 0126 B16 positive babies under one year old occurred among infants born in hospital.

As was the case with other serological types, all those infants were receiving breast milk, which is possibly the explanation for the paucity of symptoms associated with E. coli 0126 B16 in this group.

Only three other babies under one year were found to harbour E. coli 0126 B16; two had mild diarrhoea, the third had no symptoms. The three adult patients from whom this serotype was isolated all had mild diarrhoea associated with the appearance of the organism in their stools.

The relationship obtaining between patients' debility and development of symptoms is shown in Table 30.

It will be seen from the table that with the small totals involved, debilitating factors, particularly in infants and children under 12 years, were not numerous enough to merit consideration. As regards the three adults, all three were in poor physical shape. Two, one markedly senile, had advanced malignant disease; the third was an old man recovering from a herniorrhaphy operation. Diarrhoea

Table 29

The occurrence of gastro-enteritis in association with *E. coli* 0126 B16

Age Group	Total No. of patients examined	0126 B15 first isolated from faeces						Total Isolations	Incidence of *E. coli* 0126 B16 in cases examined
		On admission			After admission				
		Degree of diarrhoea			Degree of diarrhoea				
		Mild	Severe	Absent	Mild	Severe	Absent		
Under 1 month old. Born in hospital	266	-	-	-	1	0	6	7	2.6%
Premature	107	0	0	0	0	0	0	0	0%
Under 3 months	640	0	0	0	1	0	0	1	0.16%
4 to 12 months	546	0	0	0	1	0	1	2	0.37%
1 to 12 years	771	0	0	0	0	0	2	2	0.26%
Adult	1,475	0	0	0	3	0	0	3	0.2%
Totals	3,305	0	0	0	6	0	9	15	0.39%

Table 30

Clinical severity of gastro-enteritis in relation to debility

Age Group	Total Positive O126 B16	Degree of Diarrhoea	Numbers affected	Debilitating Factors					Total Debilitated
				Systemic Disease	Parenteral Infection	Recent Gastro-enteritis	Prematurity	Senility	
Adult	3	Mild	3	3	0	0	-	2	3
		Severe	0	0	0	0	-	0	
		Nil	0	0	0	0	-	0	
Non-Premature Under 3 months	8	Mild	2	1	0	0	0	-	1
		Severe	0	0	0	0	0	-	
		Nil	6	0	0	0	0	-	
4 to 12 months	2	Mild	1	0	1	0	-	-	2
		Severe	0	0	0	0	-	-	
		Nil	1	0	1	1	-	-	
1 to 12 years	2	Mild	0	0	0	0	-	-	2
		Severe	0	0	0	0	-	-	
		Nil	2	0	2	0	-	-	
Total	15		15	4	4	1	0	2	8

was present in each case for a few days. It never became severe despite the poor condition of the patients.

Severity of gastro-enteritis in relation to serological and biochemical types of E. coli 0126 B16:

It has been noted in Chapter 2 that the fifteen strains isolated were all of serological type 0126 B16 H2. The biochemical reactions were of uniform type (Table 9, Chapter 2) and, therefore, no basis was found for comparing serological and biochemical types of E. coli 0126 B16 in their ability to produce symptoms.

DISCUSSION:

The results of this investigation show that the overall incidence of the specific E. coli serotypes 0111 B4, 055 B5, 026 B6, 0125 B15 and 0126 B16 in Stobhill General Hospital was slightly greater than that of Salmonella and Shigella types which themselves are not generally regarded as being of rare occurrence in hospitals and institutions. The relatively high incidence of E. coli serotypes was, however, to some extent due to their frequent occurrence in babies under one year old who comprised approximately 40% of the 5,113 patients examined. Nevertheless in children over 1 year old and adults the incidence of specific types of E. coli was not considered to be negligible since it was found to lie between that of Salmonella and that of Shigella types.

The pathogenicity of E. coli serotypes was judged by the presence and severity of gastro-enteritis occurring in patients harbouring these serotypes. No attempt was made to correlate

occurrence of symptoms with numbers of bacteria present in stools, for two reasons. First, no culture medium was available for selective isolation of specific E. coli serotypes from other strains of E. coli. Second, despite assertions to the contrary by Shanks and Studzinsky (1952), in a site such as the intestine which has a normal bacterial flora, evidence of pathogenicity of a particular micro-organism does not depend on its being isolated in pure or almost pure culture since in the case of Salmonella and Shigella strains, whose pathogenicity is no longer in doubt, it is common experience to find these pathogens outnumbered by normal bowel flora in stool cultures made during all stages of food-poisoning or dysentery infections.

It is evident that clinical effects associated with specific serological types of E. coli vary from type to type. Table 31 summarises the clinical findings for each serotype.

Table 31

Summary of clinical findings in relation to each E. coli serotype

Strain	Numbers isolated	Patients having gastro-intestinal symptoms (per cent.)		
		Severe	Mild	None
0111 B4	158	32	41	27
055 B5	42	24	40	36
026 B6	26	15	50	35
0125 B15	60	5	25	70
0126 B16	15	0	40	60
Total	301			

With E. coli strains O111 B4, 055 B5 and 026 B6 approximately two-thirds of all patients from whom these serotypes were isolated had gastro-enteritis. It is considered that symptoms occurred in association with these serotypes with sufficient frequency and consistency to justify their being regarded as pathogens. This is in accord with current opinion on the pathogenicity of these strains (Kauffmann, 1954).

The clinical findings in respect of E. coli O125 B15 and O126 B16 are in general agreement with those of Taylor and Charter (1952) and McDonald and Charter (1956). These two serotypes were less frequently associated with disease and where symptoms were present they were usually of a mild nature. On the results of this investigation E. coli O125 B15 and O126 B16 are considered to be less pathogenic than the three former serotypes.

In the present survey serological or biochemical variants within each serotype could not be related to the development of clinical gastro-enteritis. Establishment of specific serological types of E. coli occurred most readily in the intestinal tracts of infants, but a few adults, especially those debilitated by disease or old age, were also found to harbour enteropathogenic strains. Debility from any cause could not, however, be held directly responsible for development of symptoms in either adults or infants.

It is not clear how or why certain strains of E. coli exert enteropathogenic effects. In a study of fifty-five autopsies on fatal cases of gastro-enteritis recorded by Smith (1955) local

tissue reaction in the bowel was absent and systemic dissemination of E. coli serotypes outwith the intestine did not occur. These findings indicate the possibility that the pathogenicity of certain specific serological types of E. coli may be due to their possessing an endotoxin or endotoxins not generally present in other members of the Escherichia group. This would account for the frequency with which symptoms develop in infancy since it is known that endotoxins are not strongly antitoxinogenic (Wilson and Miles, 1955) and that in infancy immunological mechanisms are imperfectly developed (Swift, 1957). It is also a well-recognised property of endotoxins that no amount of antitoxin affords protection when the dose of endotoxin exceeds a certain limit (Wilson and Miles, 1955). Thus, occurrence of gastro-enteritis in certain adults and older children might be explained on an immunological basis, for it has already been shown by Ferguson and June (1952) and by June, Ferguson and Worfel (1953) that strains of E. coli 0111 B4 and 055 B5 can produce gastro-enteritis in a normally insusceptible age group, viz., young healthy adults, provided very large doses of these organisms are given.

CHAPTER 4

PATHOGENICITY OF ESCHERICHIA COLI

II.

RELATIONSHIP BETWEEN THE ANTIBACTERIAL ACTIVITY AND THE THERAPEUTIC EFFICIENCY OF ANTIBIOTICS IN INFANTILE GASTRO-ENTERITIS

CHAPTER 4

PATHOGENICITY OF ESCHERICHIA COLI

II.

RELATIONSHIP BETWEEN THE ANTIBACTERIAL ACTIVITY AND THE THERAPEUTIC EFFICIENCY OF ANTIBIOTICS IN INFANTILE GASTRO-ENTERITIS

Antibiotic sensitivity testing of the serological types of E. coli implicated in gastro-enteritis has been the subject of a number of studies. Ferguson, Jennings and Gottshall (1951) tested thirty-two strains of E. coli 0111 B4 which were found to be sensitive, in vitro, to chloramphenicol, aureomycin, terramycin, neomycin, polymyxin and circulin. Sensitivity to streptomycin was, however, variable, only fourteen of the thirty-two strains being sensitive to this drug. Smith and Galloway (1953) studied the effects, in vitro, of aureomycin, terramycin, chloramphenicol and dihydrostreptomycin on strains of E. coli 0111 B4 and 055 B5 and found that the most inhibitory antibiotic after twenty-four and forty-eight hours' incubation was terramycin, followed by chloramphenicol, then aureomycin, and finally dihydrostreptomycin. Further, chloramphenicol, aureomycin or terramycin were not bactericidal to any strain after twenty-four hours' incubation and to only a few strains in high concentrations of the drugs after forty-eight hours' incubation. Dihydrostreptomycin, on the other hand, killed half the strains inhibited at twenty-four hours and about three-quarters at forty-eight hours.

Following on their *in vitro* studies, the latter workers performed *in vivo* tests in twenty-nine cases infected with *E. coli* 0111 B4 and in one hundred and forty infected with *E. coli* 055 B5. A variety of antibacterial agents were tested and it was found that, with the exception of sulphamezathine, streptomycin and polymyxin, the average duration of infectivity was similar for control and treated cases, namely, about sixteen days. With the three former drugs, however, this period was reduced. The authors pointed out that all the antibiotics used were of some value since, during courses of treatment, coliform organisms frequently could no longer be cultured from the faeces and that the administration of the drugs must, therefore, at least have limited the occurrence of cross infection. No attempt was made, because of the mildness of the disease, to assess clinical responses of the cases to the various antibiotics used.

In vivo investigations have also been carried out by other workers. Rogers, Koegler and Gerrard (1949) in a small series of *E. coli* 0111 B4 infections claimed clinical and bacteriological improvement with chloramphenicol. Laurell *et al.* (1951) reported good results with aureomycin in *E. coli* 0111 B4 infections. Beneficial effects from the use of chloramphenicol or one or other of the tetracyclines have been reported by Opitz (1952), Clément *et al.* (1953), Meter *et al.* (1951) and Wheeler and Wainerman (1954). Shanks and Studzinski (1952), on the other hand, were not impressed with the efficacy of chloramphenicol in shortening the course of

gastro-enteritis as judged from the period of time spent in hospital.

Most workers seem convinced that streptomycin, as distinct from chloramphenicol and the tetracyclines, is of little benefit in the treatment of gastro-enteritis, possibly because many coliform strains are insensitive to the drug (Modica, Ferguson and Ducey, 1952) or that resistant variants emerge rapidly during treatment (Alexander, Benjamin, Maslen and Roden, 1952).

It is clear from all these studies that chloramphenicol, polymyxin, the tetracyclines and, to a lesser extent, streptomycin are likely to be effective against strains of E. coli associated with gastro-enteritis. Reasonable evidence in favour of the pathogenicity of specific serological types of E. coli in gastro-enteritis would, therefore, be provided if bacteriologically positive cases could be shown to be significantly improved by the use of one or more of these potentially effective antibiotics.

The effectiveness of an antibiotic in infantile gastro-enteritis is, however, governed by two main factors. First, the humoral and cellular defences of the body are poorly developed in infancy, and it is, therefore, desirable, as Swift (1957) has recently pointed out, to use a bactericidal rather than a bacteriostatic antibiotic in this age group, particularly so in view of the large surface area of bowel wall available for harbouring pathogenic organisms. Second, it is important to obtain an adequate concentration of antibiotic throughout the whole length of the bowel, and it would seem logical, therefore, to use an antibiotic which is not

absorbed out of the bowel into the blood-stream.

During the winter of 1953-54 in the course of a controlled clinical trial carried out by Dr. A. L. Speirs, bacteriological observations were made by me on cases of gastro-enteritis harbouring the then prevalent strains of E. coli, viz., 0111 B4, 055 B5 and 026 B6. These cases were being treated with a mixture of chloramphenicol palmitate and streptomycin or with polymyxin B. By correlating bacteriological and clinical results it was hoped to obtain evidence as to the pathogenicity of the strains of E. coli in question.

The combination of chloramphenicol palmitate with streptomycin was used in order that the bactericidal action of the latter might re-inforce the bacteriostatic action of the former antibiotic. It has since been shown (Stern and Elek, 1955,) that the combination of these two antibiotics is a very effective one against E. coli. It is well-known that streptomycin is not absorbed from the intestine and is, therefore, available for local action throughout the bowel. Speirs (1954b) has shown that chloramphenicol palmitate is less readily absorbed from the gastro-intestinal tracts of infants than is the crystalline form. The antibacterial action of chloramphenicol palmitate is considered by Ross, Burke and Rice (1952) to depend on the release of the free alcoholic form of the antibiotic from the inert palmitate ester by action of the intestinal lipases. It was, therefore, felt that continuous and effective action of chloramphenicol

on the bowel flora could best be obtained by use of the palmitate form.

Polymyxin B was used because it is poorly absorbed from the gastro-intestinal tract, is bactericidal in action and is effective against strains of E. coli (Brownlee and Bushby, 1948; Brownlee, Bushby and Short, 1952).

MATERIALS AND METHODS:

Cases of gastro-enteritis admitted to Ward 42B, Stobhill Hospital, or to Ward 21, Ruchill Hospital, were allocated alternately to treated and control groups, provided that culture of their faeces yielded E. coli 0111 B4, 055 B5 or 026 B6. Patients excreting other serological types were excluded. Treated cases during the first part of the trial received a mixture of chloramphenicol palmitate and streptomycin, 20 mg. and 250 mg. per lb. body weight respectively, four times daily for six days. Later in the trial polymyxin B sulphate was substituted for the chloramphenicol-streptomycin mixture, in a dosage of 10,000 units per lb. body weight, six times daily for five days. In addition conventional supportive and dietetic measures directed at rehydration and establishment of normal feeds were instituted. Control cases received the same supportive and dietetic measures but antibiotics were withheld. Ethical reasons precluded withholding antibiotics when there was any question of a fatal outcome or when a case had already required intravenous fluids and seemed likely to relapse. Such cases were removed

from the control group and, if bacteriologically positive, were allocated to one or other of the treated groups. During the trial, clinical assessment of cases was carried out by Dr. A. L. Speirs. In the clinical assessment the following points were noted:-

1. Duration of diarrhoea.
2. Time to reach normal feeds.
3. Time to full recovery.
4. Occurrence of relapse.

The isolation and typing of strains of E. coli from patients admitted to the trial were performed by me. Bacteriological examinations of the stools of all cases were made on admission to the trial, during treatment, usually on day 2 and day 4, and after the course of treatment.

RESULTS:

The clinical results are summarised in Table 32.

A statistical analysis of these clinical results was made (Speirs 1954a). T-tests of significance were applied to the differences between the means in each group. The findings were correlated to a probability P = 0.05 and the following conclusions were reached:-

1. Duration of diarrhoea:

In the chloramphenicol-streptomycin treated group the duration of diarrhoea was significantly shorter than in the control group. The difference between polymyxin treated and control groups was not significant.

Table 32

Summary of clinical results of trial

Group	No. of Patients	Average Duration of diarrhoea (days)	Average time to reach normal feeds (days)	Average time to full recovery (days)	Relapses	Relapse Rate
Chloramphenicol Streptomycin	22	2.0	4.46	8.41	5	23%
Polymyxin	18	2.43	4.75	6.05	0	0%
Control	24	4.4	6.92	9.87	14	58%

I AM INDEBTED TO DR A.L. SPEIRS FOR PERMISSION TO REPRODUCE THIS TABLE.

2. Time to reach normal feeds:

Patients in both antibiotic treated groups took significantly shorter times to reach normal feeds than did those in the control group. The difference between the two antibiotic groups was not significant.

3. Time to full recovery:

The polymyxin treated patients took a significantly shorter time to full recovery than did the control patients. The difference between the chloramphenicol-streptomycin group and control group was not significant.

4. Relapse Rates:

X^2 tests were applied to the differences between the number of relapses. The difference between polymyxin treated and control groups was highly significant ($P < 0.01$). The difference between the chloramphenicol-streptomycin treated and control groups was significant ($0.05 > P > 0.02$). The difference between the two treated groups was not significant.

It is evident from these results that the antibiotics used were, in general, of beneficial effect in the treatment of cases in this trial.

The bacteriological results are shown in Table 33.

Table 33

Summary of Bacteriological Results

Treatment Group	Organism	No. of patients with faeces culture positive on admission to trial	No. of patients having bacteriological recurrence	Corresponding Clinical Relapse
Chloramphenicol + Streptomycin	0111 B4	13*	8	3
	055 B5	7	3	0
	026 B6	2	0	0
Polymyxin	0111 B4	13	1	0
	055 B5	5*	1	0
	026 B6	0	0	0
Controls	0111 B4	12	7	7
	055 B5	10	5	4
	026 B6	2	2	2

*Indicates one patient in the group inadequately followed up bacteriologically.

It is clear from Table 33 that bacteriological recurrences were much less frequent in the polymyxin-treated group than in either of the other two groups and that bacteriological recurrences with polymyxin were not accompanied by clinical relapse. In the chloramphenicol-streptomycin-treated group eleven (52.4%) of twenty-one patients adequately followed up bacteriologically had positive stool cultures during or immediately after treatment. This proportion is similar to that present in the control group where fourteen (58.3%) of twenty-four patients had bacteriological recurrences. In the chloramphenicol-streptomycin treated group,

however, corresponding clinical relapses were much less frequent than in the control group.

DISCUSSION:

Polymyxin appeared theoretically to be a logical drug to use in this investigation since it is effective against E. coli, is bactericidal and is not absorbed out of the intestine in significant amounts. Statistical analysis of the clinical results confirms the usefulness of polymyxin in the treatment of infantile gastro-enteritis patients. Bacteriological investigation of the cases showed that this antibiotic at the same time effectively cleared stools of the test strains of E. coli. It is reasonable to infer, therefore, that since clinical improvement accompanied successful elimination of E. coli 0111 B4, 055 B5 and 026 B6, these strains were pathogenic for the infants examined.

The combination of chloramphenicol palmitate with streptomycin, on the basis of the purely bacteriostatic action of chloramphenicol and the liability of strains of E. coli to streptomycin resistance, did not appear in theory to be such an ideal one for action against E. coli. The clinical results of this trial show, however, that exhibition of these substances was of considerable value in treating cases of gastro-enteritis. The obvious therapeutic efficiency of the combination of chloramphenicol and streptomycin in the absence of satisfactory elimination of test strains of E. coli does not, at first sight, appear to produce very

conclusive evidence for the aetiological role of these strains in infantile gastro-enteritis. Account must be taken, however, first of the frequent insusceptibility of strains of E. coli to streptomycin which would render such strains open only to the bacteriostatic action of chloramphenicol, and second, that in the absence of a satisfactory selective culture medium for specific E. coli serotypes, the isolation of these types from faeces is a qualitative rather than a quantitative test. Thus while E. coli serotypes could be isolated from faeces samples of chloramphenicol-streptomycin treated cases, these organisms, because of the bacteriostatic action of chloramphenicol, were not necessarily present in the intestine in numbers great enough to produce symptoms. This explanation for the discrepancy between clinical and bacteriological results in the chloramphenicol-streptomycin treated group is considered to be a reasonable one, which, if correct, would not invalidate the conclusions reached from results of the polymyxin treated cases, where successful bacteriological elimination with corresponding clinical improvement indicated pathogenicity of the strains of E. coli investigated.

CHAPTER 5

PATHOGENICITY OF ESCHERICHIA COLI

III.

SERUM ANTIBODY RESPONSES IN INFANTILE GASTRO-ENTERITIS

CHAPTER 5

PATHOGENICITY OF ESCHERICHIA COLI

III.

SERUM ANTIBODY RESPONSES IN INFANTILE GASTRO-ENTERITIS

Attempts have been made, notably by Smith, Galloway and Speirs (1950) and Smith (1955), to demonstrate the presence of serum antibodies to E. coli 0111 B4, 055 B5 and 026 B6 by direct bacterial agglutination tests on the sera of patients harbouring these organisms. Cooper, Walters, Keller, Sutherland and Wiseman (1955) carried out similar investigations with patients harbouring E. coli 0127. The results of these investigations have, on the whole, been disappointing in that serum agglutinins have been demonstrable only irregularly and in low titres in cases of gastro-enteritis associated with these strains of E. coli.

What is claimed to be a more sensitive method of serum antibody determination is that of haemagglutination of sensitised red blood corpuscles (R.B.C.), first described by Keogh, North and Warburton (1947, 1948) and applied to the coliform serological types 0111 B4 and 055 B5 by Neter, Bertram, Zak, Murdock and Arbeseman (1952). In this test bacterial antigens are adsorbed on to the surface of washed R.B.C. of man or other animals, the resulting sensitised cells are then challenged with serum and the presence of agglutinins to the sensitising bacteria is demonstrable by macroscopic agglutination of the sensitised R.B.C. From the investigat-

ions of Neter et al. (1952) it is apparent that such haemagglutination tests are applicable only to the demonstration of E. coli somatic antigens. Specificity tests comparing bacterial O-agglutinations with haemagglutinations indicated that the antigen adsorbed on to the R.B.C. was the same as or substantially similar to bacterial somatic antigen.

This haemagglutination technique was applied by Neter, Zalewski and Ferguson (1953) to serum specimens obtained from adult volunteers undergoing feeding experiments with E. coli 055 B5. June, Ferguson and Worfel (1953), who performed the feeding experiments, carried out bacterial O-agglutinations on the same serum specimens as those examined by Neter et al. In post feeding specimens good correlation was obtained between haemagglutination and bacterial O-agglutination but haemagglutinin titres were found to be five to twenty times higher than those of O-agglutinins.

These results suggested that use of the indirect bacterial haemagglutination test might afford an efficient method for demonstrating serum antibodies in cases of infantile gastro-enteritis and thus provide a means of assessing the pathogenicity of specific serological types of E. coli in this disease. Accordingly, after preliminary trials of the haemagglutination test, an investigation was carried out to determine serum antibody levels in infants admitted to a gastro-enteritis ward in Ruchill Hospital, Glasgow.

MATERIALS AND METHODS:

The ward selected for this investigation had non-cubicled

accommodation for sixteen babies and catered only for cases of gastro-enteritis. During the period of investigation all babies admitted to this ward had a specimen of stool or rectal swab taken on admission or within a few hours thereof. This specimen was examined for E. coli types as described in Chapter 2, Section I. OB antisera to E. coli O Groups 111, 55, 26, 86, 125, 126, 127 and 128 were used for typing. Where culture of a baby's admission specimen of faeces yielded a typeable strain of E. coli or where a baby became cross infected by a typeable strain, one colony, representative of that strain, was retained and subcultured for use in agglutination tests with that patient's serum. Where the admission specimen yielded only untypeable E. coli, one colony, considered from colonial appearances to be representative of the patient's coliform flora, was similarly retained and subcultured for use in serological tests.

Specimens of venous blood were taken from each patient on the day of admission and 10 to 20 days later. The presence of agglutinins to a patient's admission and cross infecting organisms (where the latter occurred) was determined by bacterial agglutination and haemagglutination tests on both specimens of that patient's serum. The clinical severity of each case was judged on the presence or absence of diarrhoea and dehydration.

Serological Methods:

1. Bacterial agglutinin determinations:

The presence of E. coli O and B agglutinins in serum

was respectively tested for as follows:-

'O'-Agglutinins: 0.25 ml. serial dilutions of serum in isotonic saline were mixed with equal volumes of boiled suspension of the organism under test. The mixture was incubated for 20 hours at 50°C., after which macroscopic agglutination was read.

'B'-Agglutinins: The procedure was as for O-agglutinin determination except that suspensions of live organisms were used. Macroscopic agglutination was read after incubation at 37°C. for 2 hours, followed by 18 hours at room temperature.

2. Haemagglutination determinations:

Human group O R.B.C. were washed three times with phosphate buffered saline (P.B.S.) of pH 7.8. A 48 hour agar plate culture of the strain of E. coli to be tested was suspended in 30 ml. of P.B.S., boiled for two hours and, when cool, the washed R.B.C. were added to make a final concentration of 2.5%. This mixture was incubated at 37°C. for two hours with frequent agitation, in order to adsorb bacterial somatic antigen on to the surfaces of the R.B.C. After this sensitising procedure, the R.B.C. were certrifuged free from bacteria, washed thrice as before and resuspended in P.B.S. to a concentration of 1.5%. The resulting suspension was then used as antigen in haemagglutination tests.

0.25 ml. volumes of sensitised R.B.C. suspension were mixed with 0.25 ml. serial dilutions of serum in P.B.S. and incubated for one hour at 37°C. Haemagglutination was read macroscopically after centrifuging for two minutes at

1,000 revs./min. Two control tubes were incorporated in each test. One contained the lowest dilution of serum plus washed but unsensitised R.B.C. The other contained P.B.S. plus sensitised R.B.C.

Preliminary Trial:

Haemagglutination tests for bacterial O-agglutinins were carried out in parallel with conventional bacterial O-agglutination tests using 10 strains of E. coli 0111 B4, 6 of 055 B5, 6 of 026 B6 and 10 of 0125 B15. Each strain was challenged with rabbit anti-sera prepared to these four serological types.

RESULTS:

The results of the preliminary trial are shown in Table 34. It is clear from these results that the haemagglutinin titres obtained closely paralleled those of bacterial O-agglutinins. Increased sensitivity of the haemagglutination test, which would have been indicated by higher haemagglutinin titres, was not apparent.

The clinical investigation covered the months January to March, 1956. Fifty-eight consecutive admissions were studied during this period. Seventeen infants were harbouring specific E. coli types on admission, forty-one had no typeable organisms isolated at that time, and twenty-eight babies became cross infected with a specific E. coli type while in hospital. The results are summarised in Table 35.

Table 34

Comparison of bacterial O-agglutination with haemagglutination

Strain No.	Type	O-agglutination with antisera prepared to				Indirect bacterial haemagglutination with antisera prepared to			
		0111 B4	055 B5	026 B6	0125 B15	0111 B4	055 B5	026 B6	0125 B15
1	0111 B4	6400	-	-	-	6400	-	-	-
2	0111 B4	6400	-	-	-	6400	-	-	-
3	0111 B4	3200	-	-	-	6400	-	-	-
4	0111 B4	6400	-	-	-	6400	-	-	-
5	0111 B4	6400	-	-	-	12800	-	-	-
6	0111 B4	12800	-	-	-	12800	-	-	-
7	0111 B4	6400	-	-	-	6400	-	-	-
8	0111 B4	6400	-	-	-	3200	-	-	-
9	0111 B4	3200	-	-	-	3200	-	-	-
10	0111 B4	6400	-	-	-	6400	-	-	-
11	055 B5	-	6400	-	-	-	3200	-	-
12	055 B5	-	3200	-	-	-	3200	-	-
13	055 B5	-	3200	-	-	-	6400	-	-
14	055 B5	-	3200	-	-	-	3200	-	-
15	055 B5	-	6400	-	-	-	3200	-	-
16	055 B5	-	6400	-	-	-	6400	-	-
17	026 B6	-	-	6400	-	-	-	6400	-
18	026 B6	-	-	6400	-	-	-	3200	-
19	026 B6	-	-	6400	-	-	-	12800	-
20	026 B6	-	-	12800	-	-	-	6400	-
21	026 B6	-	-	12800	-	-	-	6400	-
22	026 B6	-	-	12800	-	-	-	12800	-
23	0125 B15	-	-	-	6400	-	-	-	6400
24	0125 B15	-	-	-	6400	-	-	-	3200
25	0125 B15	-	-	-	6400	-	-	-	3200
26	0125 B15	-	-	-	6400	-	-	-	6400
27	0125 B15	-	-	-	3200	-	-	-	6400
28	0125 B15	-	-	-	6400	-	-	-	12800
29	0125 B15	-	-	-	6400	-	-	-	6400
30	0125 B15	-	-	-	3200	-	-	-	6400
31	0125 B15	-	-	-	6400	-	-	-	12800
32	0125 B15	-	-	-	6400	-	-	-	6400

Positive agglutinations and haemagglutinations are recorded as reciprocals of serum dilutions.

- = No agglutination or haemagglutination at dilution of 1 in 100.

Table 35

Summary of clinical findings in 58 babies admitted to a gastro-enteritis ward during January to March, 1956

Classification	No. of Patients	Associated Diarrhoea		
		Severe	Mild	Nil
Stool positive for typeable *E. coli* on admission	17	7	4	6
Stool negative for typeable *E. coli* on admission	41	0	7	34
Cross infected with typeable strain of *E. coli*	28	3	8	17

Nine of the fifty-eight patients investigated had serum agglutinins demonstrable to their homologous faecal strain of *E. coli*. Only three of these patients were harbouring strains of *E. coli* known to be associated with gastro-enteritis. Table 36 shows a comparison of bacterial agglutinations and haemagglutinations in serologically positive cases. The presence and severity of diarrhoea is also recorded for each case. It is apparent from these results that antibody response was exceptional and, where present, the titres obtained were generally of a low order. Antibody response involved O antibodies. No B antibodies were demonstrable. Comparison of haemagglutination with bacterial agglutination showed that in cases 6 and 7, haemagglutination tests indicated the presence of antibody which was not demonstrable by bacterial agglutination. The converse was found in cases 14 and 33. In the remaining patients the two methods gave similar results. Only three of the nine babies had

Table 36

Comparison of bacterial agglutination and haemagglutination in patients showing E. coli antibodies

Case No.	Age (months)	Organism	1st specimen of serum			2nd specimen of serum			Diarrhoea
			Bacterial Agglutination		Haemagglutination	Bacterial Agglutination		Haemagglutination	
			B	O		B	O		
6	4	U.T.	-	-	-	-	-	16	Nil
7	2	055	-	-	-	-	-	16	Mild
12	1	U.T.	-	512	256	-	8	16	Nil
14	4	U.T.	-	8	-	-	64	32	Nil
15	2	U.T.	-	128	64	-	-	-	Nil
24	2	U.T.	-	-	-	-	32	64	Mild
33	2	026	-	16	-	-	-	-	Mild
38	$4\frac{1}{2}$	0125	-	-	-	-	256	256	Nil
48	4	U.T.	-	-	-	-	8	8	Nil

Positive antibody responses are indicated as reciprocals of serum dilutions.

- = No agglutination or haemagglutination at serum dilution 1 in 8.

U.T. = Strain of E. coli untypeable with available antisera.

gastro-intestinal symptoms. No definable relationship could be demonstrated between severity of illness and the presence of serum antibodies.

DISCUSSION:

The claims of Neter et al. (1953) for the efficiency of the indirect bacterial haemagglutination test as a method of demonstrating serum agglutinins to specific serological types of E. coli were based largely on results of human adult feeding experiments in which haemagglutination appeared to be a more sensitive test than bacterial agglutination. Further work by Neter, Westphal, Luderitz, Gino and Gorzinski (1955) indicated that the haemagglutination technique was equally applicable to serological studies in infants and children, though in that investigation no direct comparison of haemagglutinations and bacterial agglutinations was made.

It is difficult to understand why haemagglutination should be considered a more sensitive method of antibody determination than direct bacterial agglutination, if, as Neter and his associates (1952) suggest, both tests demonstrate the presence of somatic antigens, and especially as it was shown that where strains of E. coli O111 B4 and 055 B5 were challenged with their homologous rabbit antisera the titres obtained by haemagglutination were closely similar to those of direct bacterial O-agglutination tests.

In the present investigation, close correlation of haemagglutinin and bacterial O-agglutinin titres has been found with rabbit antisera prepared to E. coli O111 B4, 055 B5, 026 B6 and

0125 B15. These results indicate that the two serological tests might be expected to give similar titres where O-agglutinins are present in human sera. Investigation of fifty-eight infants for the presence of serum antibodies to their homologous strains of E. coli has, in fact, shown that the occurrence of positive haemagglutinations has closely paralleled that of bacterial O-agglutinations and that haemagglutinin titres, where present, did not differ significantly from those of bacterial O-agglutinins. On this basis, haemagglutination has not been shown to be more sensitive than standard bacterial agglutination for the demonstration of serum O-agglutinins in infants.

Serological responses, generally of low order, occurred in only nine of the fifty-eight infants studied and were not confined to those infants harbouring specific serological types of E. coli. No definable relationship was evident between serological response and severity of gastro-enteritis. It was, therefore, not possible to assess, by antibody determinations, the pathogenicity of specific sero-types of E. coli in this investigation.

CHAPTER 6

PATHOGENICITY OF *ESCHERICHIA COLI*

IV.

ENTEROPATHOGENIC EFFECTS IN RABBITS OF STRAINS OF *E. COLI* ISOLATED FROM CASES OF GASTRO-ENTERITIS

CHAPTER 6

PATHOGENICITY OF ESCHERICHIA COLI

IV.

ENTEROPATHOGENIC EFFECTS IN RABBITS OF STRAINS OF E. COLI ISOLATED FROM CASES OF GASTRO-ENTERITIS

Pathogenicity of specific serological types of E. coli associated with gastro-enteritis has been successfully demonstrated experimentally in human adult volunteers by Kirby, Hall and Coackley (1950), Ferguson and June (1952) and June, Ferguson and Worfel (1953). Animal experiments directed to the same end have proved rewarding only in the hands of De, Bhattacharya and Sarkar (1956), who showed that strains of E. coli derived mainly from cases of gastro-enteritis in adults, could produce macroscopic and microscopic lesions when injected into ligated loops of rabbit bowel. The present study was undertaken in order to ascertain if the methods of De et al. (1956) were applicable to certain of those strains of E. coli commonly associated with infantile gastro-enteritis since only three such strains were included in these workers' study.

MATERIALS AND METHODS:

Adult rabbits, approximately 5 lbs. (2.3 kg.) in weight, were used. Each test animal was given water only for 18 hours, then anaesthetised with an intra-peritoneal injection of sodium pentobarbitone (60 mg./5 lb. weight) followed by open ether. The abdomen was opened and a loop of small intestine 4" to 6" long was

isolated by silk ligatures. Into the lumen of this loop was injected 2.0 ml. of a 24 hour peptone water culture (pH 8.4) of the organism under test. The abdomen was then closed and the animal allowed to recover. Twenty-four hours after operation, the rabbit was killed by chloroforming. The isolated bowel loop was examined macroscopically for congestion and distension and the contents of the loop cultured for E. coli. The bowel was then fixed in 10% formal saline and prepared for histological examination. Initially, experiments were restricted to one per animal. Later, for the sake of economy, this was increased to two per animal using bowel loops at least 9" apart.

Twenty-three strains of E. coli of the serological types commonly present in cases of infantile gastro-enteritis were investigated. Nine strains were isolated from babies during the acute stage of severe gastro-enteritis, i.e., gastro-enteritis necessitating intravenous therapy. Six strains were derived from babies having mild gastro-enteritis and seven from babies having no symptoms. One further strain was obtained from an adult having acute vomiting and diarrhoea. Control experiments were carried out using four serologically untypeable strains of E. coli isolated from healthy babies. Seven more control experiments were performed to ascertain the effects of ligation alone and ligation plus injection of sterile peptone water at pH 8.4.

RESULTS:

The results are given in full in Table 37 and are summarised in Table 38. It became apparent early in the experiments that gross appearances did not strictly correspond to those of microscopy. Thus, from Table 37, in Control 1 where ligation only was performed, the macroscopic appearances suggested a much more definite lesion of the bowel wall than was demonstrable histologically. In Controls 2, 3, 6 and 7, on the other hand, collapsed bowel of normal appearance showed desquamation and necrosis of the tips of the villi microscopically. Likewise, in experiments T.11, 13, 15, 16, 17, 18 and 28 histological examination showed deviations from normal bowel pattern in the absence of marked naked eye changes.

Histological findings in the eleven control experiments showed essentially normal bowel structure without oedema or cellular infiltration in the submucosa (Figure 1). Occasionally desquamation of the superficial epithelium of the villi was seen. This appearance was regarded as being non-specific and probably due to experimental trauma. Experiments involving four strains of E. coli 026 B6, two of 055 B5, two of 0111 B4 and one of 0125 B15 (T.1 to T.10 inclusive) showed similar histological findings to those present in control experiments. It will be seen from Table 38 that of the nine individual strains studied in this group, six were isolated from patients having no diarrhoea, two were from cases of mild gastro-enteritis and one from a severely ill baby. In the nine experiments, T.11, 12, 13, 15, 16, 17, 18, 19 and 20, the

TABLE 37

Summary of Experiments and Results

Experiment	Material Injected	Associated Clinical Severity in Patient from whom Organism isolated	Post Mortem Investigations on Test Animals		
			Culture of Bowel Loop Contents.	Macroscopic Appearances of Ligated Bowel Loop	Histology of Bowel Wall
Control 1	None. Bowel ligated only	-	E. coli not isolated	Moderate congestion and distension.	No gross congestion. No cellular infiltration but scattered polymorphs present in lumen.
Control 2	Untypeable Strain of E. coli	No symptoms	Moderate growth of E. coli	Collapsed and of normal appearance	Desquamation and necrosis tips of villi. No cellular reaction.
Control 3	Untypeable Strain of E. coli	No symptoms	Scanty growth of E. coli	Collapsed with very slight uniform congestion.	Desquamation of superficial epitholium of villi. Cellular reaction slight, mainly round cells.
Control 4	Untypeable Strain of E. coli	No symptoms	Heavy growth of E. coli	Bowel contains opaque fluid. No gross distension.	Normal bowel.
Control 5	Untypeable Strain of E. coli	No symptoms	Moderate growth of E. coli	Normal bowel	Normal bowel.
Control 6	2 ml. of sterile peptone water injected.	-	E. coli not isolated	Slight uniform congestion. No distension.	Necrosis and loss of tips of villi but no inflammatory reaction or oedema present.
Control 7	2 ml. of sterile peptone water injected.	-	E. coli not isolated	Slight congestion only.	Necrosis and loss of tips of villi but no inflammatory reaction or oedema present.
Control 8	2 ml. of sterile peptone water injected	-	E. coli not isolated	Bowel collapsed	Normal Bowel.
Control 9	2 ml. of sterile peptone water injected.	-	E. coli not isolated	Bowel collapsed	Normal Bowel.
Control 10	2 ml. of sterile peptone water injected	-	E. coli not isolated	Normal Bowel	Normal Bowel.
Control 11	2 ml. of sterile peptone water injected	-	E. coli not isolated	Normal Bowel	Normal Bowel.
T.1	055 B5 9737/2	Mild gastro-enteritis	055 B5 isolated	Normal Bowel	No congestion or cellular infiltration. Mucosa intact.
T.2	055 B5 2737/2	Mild gastro-enteritis	055 B5 isolated	Normal Bowel	Normal Bowel.
T.3	0125 B15 292/3	No symptoms	0125 B15 isolated	Normal Bowel	Normal Bowel.
T.4	0111 B4 2712/4	Failure to thrive. No gastro-enteritis	0111 B4 isolated	Normal Bowel	Normal Bowel.
T.5	026 B6 10503/6	No symptoms	026 B6 isolated	Some distension and congestion	Extensive desquamation of epitholium. No cellular reaction.
T.6	026 B6 10510/6	No symptoms	026 B6 isolated	Distension with slight congestion	Desquamation of epitholium. No cellular reaction.
T.7	0111 B4 3410/6	No symptoms	0111 B4 isolated	Slight congestion. No distension.	Normal Bowel.
T.8	026 B6 9904/6	Mild gastro-enteritis	026 B6 isolated	Slight congestion. No distension.	Slight desquamation of epitholium. Otherwise normal.
T.9	026 B6 14436/6	Severe gastro-enteritis	026 B6 isolated	Normal Bowel	Normal Bowel.
T.10	055 B5 13258/6	No symptoms	055 B5 isolated	Normal Bowel	Normal Bowel.
T.11	0125 B15 2799/2	Severe gastro-enteritis	0125 B15 isolated	Slight uniform congestion. No swelling.	Bowel structure normal but mucosa and villi are oedematous.
T.12	0111 B4 2857/3	Severe gastro-enteritis	0111 B4 isolated	Marked distension with patchy congestion and fibrinous exudate on serous surface.	Mucosa intact but villi oedematous.

T.13/

Experiment	Material Injected	Associated Clinical Severity in Patient from whom Organism isolated	Post Mortem Investigations on Test Animals		
			Culture of Bowel Loop Contents	Macroscopic Appearances of Ligated Bowel Loop	Histology of Bowel Wall
T.13	0111 B4 11220/2	Severe gastro-enteritis	0111 B4 isolated	Bowel collapsed with slight surface congestion	Oedema of mucosa and early desquamation. No cellular reaction present
T.14	0111 B4 6483/2	Mild gastro-enteritis	0111 B4 isolated	Bowel distended and gangrenous	Oedema of mucosa with areas of haemorrhage, ulceration and necrosis
T.15	055 B5 2118/6	Mild gastro-enteritis	055 B5 isolated	No distension but slight uniform congestion	Slight oedema of submucosa
T.16	055 B5 2118/6	Mild gastro-enteritis	055 B5 isolated	No distension but slight uniform congestion	Slight oedema of submucosa
T.17	0111 B4 4204/6	Mild gastro-enteritis	0111 B4 isolated	Slight congestion. No distension.	Slight oedema of submucosa
T.18	0111 B4 4204/6	Mild gastro-enteritis	0111 B4 isolated	Slight congestion. No distension.	Slight oedema of submucosa
T.19	0111 B4 4596/6	Severe gastro-enteritis	0111 B4 isolated	Congestion but no marked distension.	Moderate congestion and marked oedema of submucosa
T.20	0111 B4 4596/6	Severe gastro-enteritis	0111 B4 isolated	Bowel congested.	Marked oedema of submucosa
T.21	0111 B4 3884/3	Severe gastro-enteritis	0111 B4 isolated	Bowel swollen, congested and gangrenous	Congestion, focal ulceration of mucosa and heavy polymorphonuclear infiltration.
T.22	026 B6 2713/4	Adult gastro-enteritis	026 B6 isolated	Bowel swollen, congested and gangrenous	Focal lesions of mucosa. Large aggregations of macrophages and polymorphs projecting into lumen of bowel. Fibrotic exudate present on serosa.
T.23	0111 B4 2821/6	Severe gastro-enteritis	0111 B4 isolated	Animal moribund. Bowel distended with patchy haemorrhages and fibrinous adhesions	Very acute reaction with necrosis of villi, haemorrhages and focal polymorphonuclear infiltration
T.24	0111 B4 2821/6	Severe gastro-enteritis	0111 B4 isolated	Bowel loop distended and gangrenous	Animal found dead on morning after operation. Histology not practicable.
T.25	0111 B4 2821/6	Severe gastro-enteritis	0111 B4 isolated	Bowel loop distended and gangrenous	Animal found dead on morning after operation. Histology not practicable.
T.26	055 B5 8004/1	Severe gastro-enteritis	055 B5 isolated	Bowel grossly distended and almost gangrenous	Oedema of mucosa with areas of haemorrhage, ulceration, necrosis and focal polymorphonuclear infiltration.
T.27	0111 B4 3777/6	No symptoms	0111 B4 isolated	Moderately distended and congested.	Extensive necrosis with loss of epithelium and focal polymorphonuclear reactions.
T.28	0111 B4 4253/6	Mild gastro-enteritis	0111 B4 isolated	Slight congestion. No distension.	Extensive desquamation from villi and necrosis. Foci of acute inflammation present.
T.29	026 B6 11735/6	Severe gastro-enteritis	026 B6 isolated	Marked distension and congestion.	Desquamation of epithelium with ulceration, necrosis and polymorphonuclear infiltration.

Table 38

Correlation of the clinical effects of specific serological types of *E. coli* on human hosts with the histopathogenic effects of these organisms on ligated rabbit intestine

Clinical Manifestations in patients	Number of Patients	Serotype of *E. coli*	Histopathogenic Effects		
			None	Mild	Severe
No symptoms	2	026 B6	2	-	-
	1	055 B5	1	-	-
	3	0111 B4	2	-	1
	1	0125 B15	1	-	-
Mild symptoms	2	026 B6	1	-	1
	2	055 B5	1	1	-
	3	0111 B4	-	1	2
Severe symptoms	2	026 B6	1	-	1
	1	055 B5	-	-	1
	5	0111 B4	-	3	2
	1	0125 B15	-	1	-

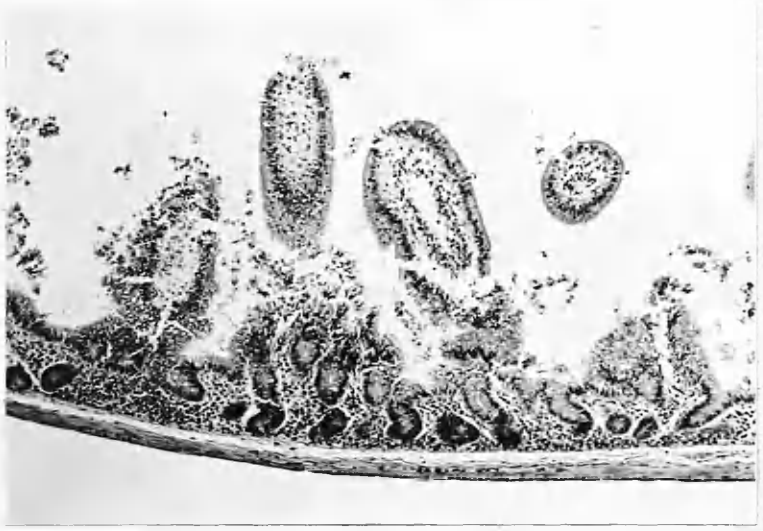

Figure 1 - Rabbit intestine 24 hours after ligation and injection with sterile peptone water. No gross abnormality is seen in the mucosa. H. & E. x 80.

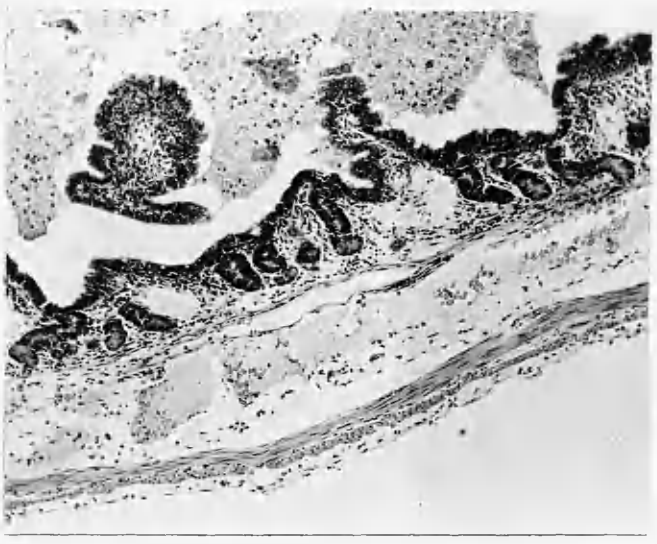

Figure 2 - Rabbit intestine 24 hours after ligation and injection with a strain of E. coli 055 B5 from a patient with mild gastro-enteritis. There is gross oedema in the mucosa and submucosa but no cellular reaction is present. H. & E. x 80.

presence of oedema in the villi and submucosa was the most marked microscopic feature (Figure 2). This reaction was regarded as being a specific histological change of mild type. It was observed with four strains of E. coli 0111 B4, one of 055 B5 and one of 0125 B15. All six of these strains were isolated from cases of gastro-enteritis (Table 38).

In eight experiments (T.14, 21, 22, 23, 26, 27, 28 and 29) a severe type of tissue reaction was encountered. This reaction was characterised by necrosis and ulceration of the mucosa with marked infiltration of polymorphonuclear leucocytes in the mucosa and muscle coats (Figure 3). Five strains of E. coli 0111 B4, two of 026 B6 and one of 055 B5 gave this type of histological lesion. With one exception these strains were derived from patients suffering from mild or severe gastro-enteritis (Table 38).

In experiments T.1 and 2, T.15 and 16, T.17 and 18, T.19 and 20, and T.23, 24 and 25, where repeated observations were made on strains of E. coli, Table 37 shows that pathogenic changes were reproducible in every case.

DISCUSSION:

The results of this investigation show the existence of good correlation between experimental lesions produced by specific serological types of E. coli and the association of these same strains with gastro-enteritis in the human subject. Thirteen (81.25%) of sixteen such strains examined (Table 38) produced lesions

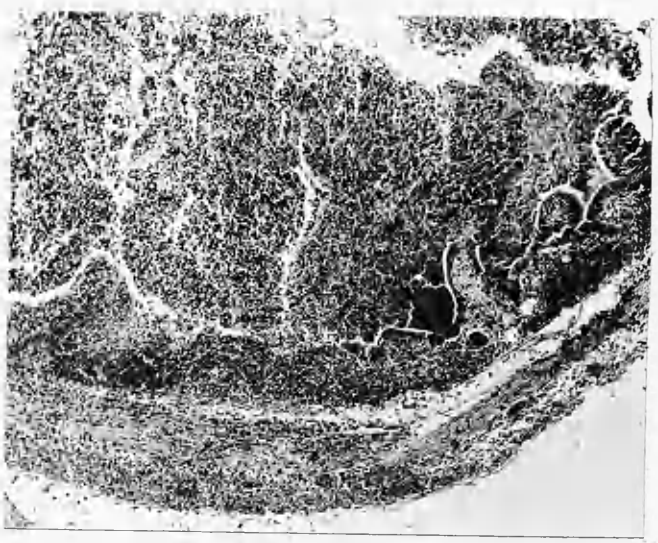

Figure 3 - Rabbit intestine 24 hours after ligation and injection with a strain of E. coli O111 B4 isolated from a baby with severe gastro-enteritis. There is extensive necrosis of the mucosa with gross cellular reaction which affects the whole thickness of the bowel wall. H. & E. x 80.

in ligated rabbit bowel loops distinct from those produced by serologically similar and control strains isolated from symptomless babies. The results compare favourably with those reported by De *et al.* (1956) in similar experiments, where thirteen (65%) of twenty strains obtained from cases of acute gastro-enteritis gave rise to experimental lesions in rabbits.

Of the seven typeable and four untypeable strains investigated from asymptomatic babies, only one (9%) produced what was considered to be a significant histological change experimentally. Again this compares favourably with the results of De *et al.* who found that three (15%) of twenty strains from healthy carriers gave rise to discernible changes in rabbit bowel.

Although my results show a close similarity to those of De *et al.* the lesions produced in my experiments differed in some respects from those reported by the latter workers. Attention has already been drawn to the unreliability of gross appearances as a criterion of pathogenic change. Histological lesions of the oedematous type described by De *et al.* were present in experiments involving six strains of *E. coli* but a much more acute ulcero-necrotic lesion with aggregations of polymorphonuclear leucocytes occurred with a further eight strains. The appearances of the latter reaction are consistent with its being a more severe version of the former and not necessarily an entirely different type of lesion.

The degree of reaction observed in experimental animals

did not give any clear indication of the severity, as distinct from the presence, of gastro-enteritis in the human subject (Table 38). When account is taken of the many variable factors influencing host resistance to infection it would have been surprising to have been able to correlate actual severity of illness with distinctive experimental lesions.

Nevertheless the results of this investigation show that strains of E. coli isolated from cases of gastro-enteritis produce enteropathogenic effects in rabbits with sufficient frequency and consistency to suggest that an experimental method is now available for estimating the pathogenicity of strains of E. coli for human hosts. The good correlation obtained between experimental results and clinical findings also goes far to establishing a definite aetiological relationship of certain E. coli serotypes to human gastro-enteritis.

Acknowledgement

I am indebted to Dr. G. B. S. Roberts, Ruchill Hospital, Glasgow, for technical assistance in the animal laparotomies.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

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The infective nature of epidemic infantile gastro-enteritis is no longer seriously disputed. It is only within the past decade, however, that any wide credence has been given to the role of Escherichia coli as an important aetiological agent of the condition. Since 1944 overwhelming evidence implicating E. coli has been provided by the isolation of certain specific serological types of the organism from high proportions of cases in nosocomial epidemics of the disease, with clinical and bacteriological evidence of case to case spread. Good clinical responses to antibiotic therapy directed against E. coli have also been recorded and feeding experiments directed to the experimental production of gastro-enteritis in human volunteers have met with considerable success. Although the demonstration of antibody responses in the sera of affected individuals and the reproduction of disease by feeding experiments in laboratory animals have been less successful, exception to the aetiological role of E. coli has seldom been taken on these grounds. Dissident views have been expressed principally because of the frequent isolation of specific E. coli serotypes from babies, in the absence of gastro-enteritis. This standpoint is illogical in the light of experience with the recognised intestinal pathogens of Salmonella and Shigella groups where it is common to isolate these organisms from the stools of asymptomatic

patients.

While definitive work on the enteropathogenic nature of strains of E. coli has principally concerned the serotypes 0111 B4 and 055 B5, other serological types have, since the commencement of this study, been associated with cases and epidemics of infantile gastro-enteritis. Kauffmann (1954) has voiced the general opinion that the serotypes 026 B6 and 086 B7 should, like the former two serotypes, be regarded as aetiological agents of the disease. The pathogenicity of more recently recognised serological types has not, however, been so well established.

The results of the present study, in which approximately two-thirds of all patients found to harbour serotypes 0111 B4, 055 B5 and 026 B6 had or developed clinical gastro-enteritis, confirm current opinion on the pathogenicity of these strains. E. coli 086 B7 was not encountered during the hospital survey but two other serotypes, viz., 0125 B15 and 0126 B16, did occur. These two serotypes were less often associated with severe gastro-intestinal symptoms and, on this basis, can only be regarded as less pathogenic than E. coli 0111 B4, 055 B5 and 026 B6.

It is unfortunate that a medium for the selective isolation of potentially enteropathogenic strains of E. coli from stool samples has not yet been evolved. Existing culture methods do not readily lend themselves to efficient use in a busy routine laboratory, since they depend to a great extent on the patience of the operator, which tends to vary inversely with the number of cultures

to be dealt with. There can be little doubt that specific serological types of E. coli from cases of infantile gastro-enteritis and from asymptomatic carriers are thus frequently missed. The epidemiological and prognostic aspects of this deficiency are of much more serious moment than any failure to provide a quantitative estimate of the numbers of specific E. coli serotypes on culture plates. Isolation of these organisms in pure or almost pure culture from cases of gastro-enteritis, again by analogy with Salmonella and Shigella infections, is not considered to be a specific requirement for their pathogenicity.

Study of fermentation reactions of strains of E. coli isolated during the survey suggested that use of the glucoside salicin might provide a more efficient means of differentiating potentially enteropathogenic strains of E. coli from many of the other organisms commonly met with in faeces. The results with Salicin Bromthymol-blue Agar medium did in fact indicate that use of this medium was more efficient and less time-consuming than MacConkey medium for the primary isolation of specific E. coli serotypes. For these reasons it is considered to be an improvement on MacConkey for routine laboratory use.

Most hospital bacteriologists are aware of the frequent occurrence of apparently infective nosocomial diarrhoea from which no pathogenic or potentially pathogenic micro-organism can be isolated. The results of the hospital survey show that the incidence of specific serological types of E. coli, even in patients over

1 year old, was not inconsiderable compared with that of Salmonella and Shigella strains. Moreover, of the forty-seven adults and older children found harbouring typeable strains of E. coli, seven (14.9%) had severe, and twenty-one (44.7%) had mild gastro-enteritis. So that, as with infants, almost two-thirds of the patients with positive stool cultures had clinical evidence of gastro-enteritis. These results suggest that epidemiological investigation of institutional outbreaks of diarrhoea should include search for specific serological types of E. coli.

In the hospital survey, factors thought likely to be related to the severity of gastro-enteritis were investigated, but neither special characteristics of the E. coli strains nor debilitating factors in patients could be shown to have any definite prognostic significance. There was, however, some indication that establishment of specific E. coli serotypes in the bowel was more common in debilitated patients, especially in those over 3 months old. To this extent, therefore, debility appeared to increase susceptibility to infection.

As regards specific antibacterial treatment of infantile gastro-enteritis, several factors govern the successful use of antibiotics in this condition. The principles involved have been outlined in an excellent paper by Swift (1957). Because of the immaturity of the immunological mechanisms in infancy, Swift has stressed the importance of using bactericidal rather than bacteriostatic agents in this age group. Moreover, it is desirable to

maintain effective concentrations of antibiotic at the site of infection, i.e., in the bowel lumen, in the case of infantile gastro-enteritis. Thus, for efficient treatment it would appear to be necessary to use bactericidal antibiotics which are effective against *E. coli* and are not absorbed from the bowel. Polymyxin B fulfilled these requirements to a greater extent than other antibiotics in common use, and the results of polymyxin therapy in the clinical trial confirmed its theoretical promise.

Attempts by various workers to demonstrate serum antibody responses to specific *E. coli* serotypes in cases of infantile gastro-enteritis have not been consistently successful. This is unfortunate, since it is recognised (Wilson and Miles, 1955) that an accurate assessment of the relationship of a suspected micro-organism to a given disease can be made by demonstration of specific antibodies, in abnormally large amounts, in the blood of affected individuals. In the present study, it was found that use of the indirect bacterial haemagglutination technique of Neter *et al.* (1952) still failed to provide clear evidence, in gastro-enteritis patients, of the presence of serum antibodies to *E. coli*. No indication of the pathogenicity of these organisms was, therefore, obtainable by this means. It is perhaps not altogether surprising that antibodies are not regularly demonstrable in infantile gastro-enteritis, first, because of the immaturity of those patients most often affected and, second, because of the failure to show, in autopsies

of fatal cases, invasion of the host's tissues by E. coli

Investigation of the pathogenicity of specific serological types of E. coli by experiments on laboratory animals has, until now, been unrewarding. By using the method of De et al., 1956, it was, however, possible for histopathogenic changes to be produced in rabbit intestine by E. coli types 0111 B4, 055 B5, 026 B6 and 0125 B15. The good correlation shown between the presence of gastro-enteritis in patients, and the lesions obtained in experimental animals, indicates that the method is a suitable one for demonstrating enteropathogenic properties of E. coli strains.

Taken as a whole, the results of this investigation present further evidence that certain serological types of Escherichia coli are important aetiological agents of epidemic infantile gastro-enteritis. This concept is in accord with the changing characteristics of the disease, which has, since the beginning of the twentieth century, become less of a scourge to the infant population in this country. On the assumption that the disease was at one time food-borne, the decline of epidemic infantile gastro-enteritis can be logically related to generally improved standards of hygiene in the past fifty years. In this connection, the observations of Thomson (1956), on the occurrence of potentially enteropathogenic varieties of E. coli in cows' milk suggest that this was probably a very important source of infection. In Britain, unheated cows' milk is no longer fed to infants. To-day, infantile gastro-enteritis is a disease of infant communities in hospitals and institutions. Case to

case spread in hospitals has been clearly demonstrated by Rogers (1951) to take place as a result of the gross environmental contamination by cases harbouring specific E. coli serotypes.

Within the last decade, however, even in hospitals and institutions the occurrence of the disease has become less frequent and its effects less dangerous. This can be directly related to general acceptance by paediatricians and bacteriologists of the enteropathogenic potentialities for infants of certain specific serological types of E. coli. The importance of this new attitude to special serotypes of E. coli is reflected in the work of Jameson et al. (1954), who successfully eradicated gastro-enteritis from an infant hospital ward, over a period of sixteen months, by taking steps to exclude these serotypes from the ward. Moreover, antibiotics are now generally available for use against E. coli. While it has already been pointed out that some antibiotics are more likely to be effective than others in the treatment of infantile gastro-enteritis, Smith and Galloway (1953) have shown that several antibiotics in common use, notably the tetracyclines and chloramphenicol, significantly inhibit the coliform flora of the intestine and, therefore, to some extent must limit cross infection by specific serotypes. This has been confirmed experimentally in the case of chloramphenicol by Todd and Hall (1953). From the results obtained with polymyxin in the present investigation, and from those recorded by Rogers et al. (1956) with neomycin, it is also apparent that rational and effective treatment of the disease with antibiotics

is now possible. Thus, the experimental studies of the past decade, and the behaviour of the disease during this period, confirm the importance of Escherichia coli as an aetiological agent of epidemic infantile gastro-enteritis.

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